MICROBES AND ULTRAMICROBES

AN ACCOUNT OF BACTERIA, VIRUSES AND THE BACTERIOPHAGE

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PREFACE

THE aim of this book is to provide workers and students in Biology with an adequate account of the ultramicroscopic agents of disease and of transmissible bacterial lysis; that is, of the viruses and the bacteriophage. These I have classed together as 'Ultramicrobes', deriving the term from the familiar adjective 'ultramicroscopic', and braving the disapproval of linguistic purists. This group of elusive entities may be defined as 'agents below or on the borderline of microscopic visibility, which cause disturbance of the functions of living cells and are regenerated in the process'.

Since the bacteriophage is intimately connected with the life, death and variation of bacteria, no account of it would be complete without a preliminary survey of these subjects, in so far as they are relevant to the purpose.

In the study of microbes and ultramicrobes many problems of a wider interest arise, especially in the field of genetics, which extend beyond my range of competence; and for this reason a short commentary by Mr. de Beer is included as an appendix.

My general policy has been to make few references to individual researchers, but always to provide access to the latest comprehensive treatises and bibliographies. Pioneer researchers and the authors of specially significant pieces of recent work are mentioned from time to

time, but without any aim at completeness or consistency. Technical terms have been avoided as much as possible, and a Glossary of the few that are not explained in the text will be found at the end of the book.

A. D. G.

OXFORD
June, 1931

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PART III

MICROBES AND ULTRAMICROBES

PART I

BACTERIA AND THEIR VARIATION

§ 1. THE STRUCTURE AND FUNCTIONS OF BACTERIA

Structure

BACTERIA are minute cells which, so far as we know, consist of semi-liquid protoplasm surrounded by a flexible protoplasmic membrane devoid of cellulose and chitin. The membrane is delicate and thin in young, actively growing cells, and tougher and more rigid in old ones. Bacteria contain no nucleus comparable with the cell-nuclei of protozoa or higher creatures, but evidence is accumulating that they are not altogether devoid of nuclear apparatus. The study of intracellular structures in cells 0·5 μ or less in diameter is beset with technical difficulties, and it is exceedingly hard to judge of the value of the various observations. Two recent pieces of work, however, by Stoughton and Barnard respectively, have reinforced the numerous earlier reports of an elementary nuclear structure in certain species. Stoughton claims to have seen in living unstained cultures of *Pseudomonas malvacearum* rather

ill-defined intracellular bodies which divide as the cell undergoes fission, one-half passing into each new cell. This confirms his own and earlier observations made with methods of vital staining. Barnard has photographed various bacteria (*Bacillus megatherium, Staphylococcus*, etc.) with ultraviolet light, and shown that in some, particularly when undergoing fission, spherical

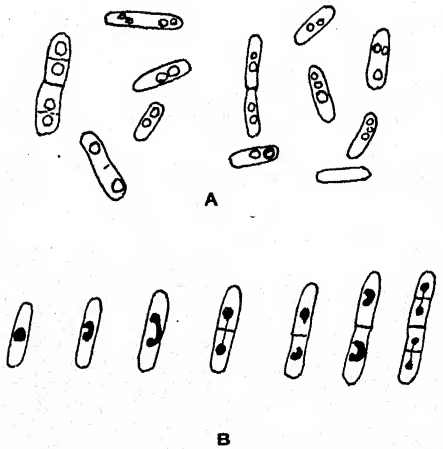

FIG. 1.—Intracellular Structures resembling Nuclei

A, As photographed by ultraviolet light. Diagrammatic; after Barnard. B. megatherium. *B*, Stained cells arranged in artificial sequence to show alleged division of nucleus. B. malvacearum. (After Stoughton.)

intracellular structures can be demonstrated often in a state of apparent division (see Fig. 1).

Dobell and others have shown that the cell contains chromatophilic granules which are not very easy to demonstrate, and the aggregation of these granules into a 'nuclear' mass before division has been described by several workers in recent years. Further,

if Stoughton's observations can be trusted, these masses take part in cell-division.

Piecing together the fragments of evidence, we can take the provisional view that bacteria have an intracellular apparatus analogous to a nucleus, but differing in being dispersed as granules throughout the protoplasm in the resting stage of the cell. It appears that the granules aggregate in a mass as the cell reaches its full growth, and that this mass plays a part in fission and the formation of new cells.

For further details and an account of earlier work the reader is referred to Vol. I of the *System of Bacteriology* (Medical Research Council), 1930.

Apart from these granules, many bacteria contain larger and more obvious spherical bodies composed of lipoids or of a special substance known as volutin, both of which are generally considered to be reserves of food. Dead bacterial cells often show chromatophilic granules resulting from protoplasmic disintegration, which are only of interest from having often been erroneously taken for reproductive bodies (gonidia).

The form of bacteria (Fig. 2) may be that of a sphere or spheroid (*Coccus*): a short or long straight cylinder (*Bacillus*, *Bacterium*, etc.): or a cylinder bent in corkscrew form (*Spirillum*, *Vibrio*). These forms are mere approximations, for in life the numerous species show considerable minor differences. Some rods, for instance, are tapered, like *Fusiformis*, others ovoid, irregularly bent, and so on. Moreover, as we shall see later, great variations may occur in a single population of any species.

The size of the majority of bacteria is from 1 to 10 μ in length and from 0·25 to 1 μ in breadth. Much larger organisms (e.g. *B. bütschlii*, 50 $\mu \times 5\,\mu$) have been described as bacteria, but may belong to another order.

The smallest visible organisms will be dealt with in the chapter on Viruses.

Spores (Fig. 3) are a special form of cell, originating singly in vegetative cells and characterized by a very stout membrane and high fat- and low water-content, which together give them a very high resistance to a

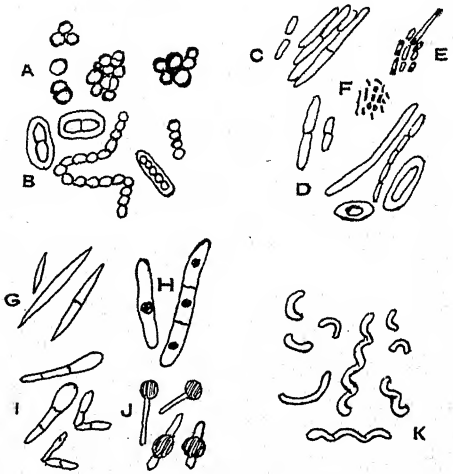

FIG. 2.—Forms of Bacteria

A. Staphylococcus. Micrococcus. *B*, Streptococcus. *C*, Bacterium. *D*, Various rods, capsulated and uncapsulated. *E*, Pfeifferella, etc. *F*, Haemophilus, etc. *G*, Fusiformis. *H*, Bacillus. I, Corynebacterium. *J*, Clostridium. *K*, Vibria; spirillum.

variety of physical and chemical agents, such as heat, light, and antiseptic substances.

Only a small proportion of bacterial genera bear spores (*Bacillus* and *Clostridium*), by virtue of which they can survive for indefinite periods in the outside world under all sorts of conditions. Most of the species are saprophytes, but the vegetative forms of some (e.g.

Bac. anthracis and *Clost. tetani*) are pathogenic. Spore formation is not a mode of multiplication, since not more than one spore is formed by the cell.

Flagella are possessed by a large number of the rod-shaped and spiral organisms. They are single or multiple protoplasmic processes of extreme tenuity, sometimes just visible by dark-ground illumination, and

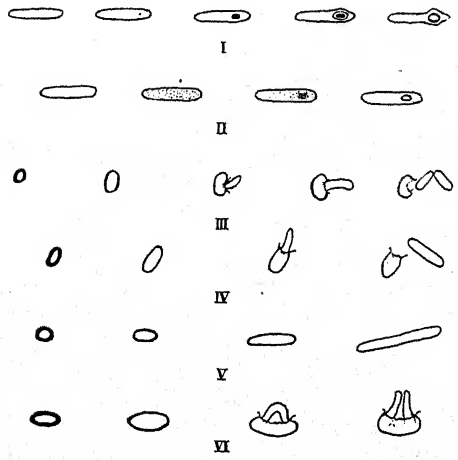

FIG. 3.—Spore-formation (I and II) and Germination (III to VI) in various species of Bacteria

needing special process for staining. Their chemical structure or antigenic composition (see Glossary) differs from that of the body of the cell. Although rotatory locomotion ('motility') of the cell is their most obvious effect, they should properly be regarded as nutritive organs which by constant undulation perpetually change the fluid in which the organism is bathed. Absence of flagella, however, whether by normal constitution or

modification, does not appear to reduce the efficiency of a race.

Capsules are variable structures external to the cell-membrane, and needing special staining methods to reveal them. Some species, such as the *Pneumococcus* and *Bact. mucosum capsulatum*, are normally capsulated; others, like *Streptococcus*, can form capsules exceptionally in adverse conditions, such as in the living tissues of man and animals. By this means the microbe is protected from the antibodies in the tissue-fluid, and its infecting power is thereby increased. Recent biochemical work in America has shown the capsular substance of *Pneumococcus* to be largely composed of polysaccharides, and that the variety of the chemical constitutions of these substances explains the 'antigenic' differences of the races or 'types'. Other bacteria, not obviously capsulated, also have similar 'specific soluble substances'.

False capsulation frequently appears in certain bacteria which can, on occasion, secrete a slimy or mucoid substance in which they remain embedded.

Staining Reactions. Nearly all bacteria take up basic dyes readily. Acid dyes stain them faintly, and hæmatoxylin hardly at all. A most valuable differentiation into two great groups of genera is given by Gram's stain (Methyl violet,—followed by iodine; decolorization with ethyl alcohol or acetone). Gram-positive organisms are those that retain the stain, owing to the formation of an insoluble dye-compound in the ectoplasmic layer (see Specification of genera, p. 18). The important *Mycobacterium tuberculosis*, in common with the microbe of leprosy and a few saprophytic species, has the property of retaining carbol-fuchsin stain during decolorization with 25 per cent mineral acid. This 'acid fastness' is of the utmost value in

identifying *Myc. tuberculosis* in various pathological materials.

Antigenic Structure. Since microbes are small and chemistry crude, bacteriology has had to content itself with indirect methods of biochemical analysis. The body of animals has the most remarkable power of analysing complex foreign nitrogenous substances injected into it, and elaborating compounds with a specific physico-chemical affinity for the injected substance. Thus, a rabbit injected with a suspension of *Bact. typhosum* elaborates in the course of a week or less a number of 'antibodies' capable of combining with the various 'antigèns' of which the bacterium is composed. There are several different reactions for detecting and measuring the concentration of the antibodies in the blood-serum, and of these the agglutination test is the most important for our purposes.

If progressive dilutions of the serum are mixed with a constant quantity of a suspension (alive or dead) of the species injected, clumps will gradually form in all the tubes in which the serum is strong enough, and the dilution of serum of the last tube that shows clumping (agglutination) gives the measure of the strength or 'titre' of the serum.

Bacteria chemically unrelated to the injected species give no reaction; related ones react partially. Finer analysis shows that the serum contains several antibodies, each acting on a special component (antigen) of the bacterial body. These several antibodies can be removed from the serum by 'absorption' with the separate antigens. Thus, if a related species, or a variant of the same species, possesses only one of the antigenic components, treatment of the serum with a suspension of this organism, which is then removed by centrifugalization, will eliminate the one antibody, leaving the others intact.

This method provides us with a means of antigenic analysis which has yielded much information about the structure, relationships and variation of bacterial species.

It has been proved in the case of the *Pneumococcus* that the specificity of the antigen of a given variety depends on the chemical structure of a polysaccharide in the capsular material. The same appears to be true also in many other species.

Cultivation and Nutrition

Pure cultures are obtained by diluting the matter containing the bacteria with sterile water or physiological salt solution and spreading a small drop on a large surface of nutrient gellified medium. The cells are thus separated, and each will grow into a colony of many thousand individuals.

Carbon is extracted by most bacteria from preformed carbon-compounds ranging from formic acid to carbohydrates and proteins. A few organisms, however, can utilize CO_2. One pathogenic species (*Brucella abortus*, which causes contagious abortion in cattle, and an undulant fever in man) will only grow, when first isolated, if it is provided with a good deal of CO_2, though it usually adapts itself after a time to life in ordinary air.

The fermenting power of a species for a range of carbohydrates and alcohols provides one of our best differential tests. For instance, the power of producing acid and gas from lactose distinguishes *Bact. coli* from a large group of pathogenic bacteria including *Bact. typhosum* and *Bact. dysenteriae*, which are otherwise very like it.

Nitrogen is obtained by most species from organic compounds such as amino acids. A few only can use atmospheric nitrogen (e.g. *Azotobacter*).

Oxygen. The majority of bacteria are aerobes, i.e.

they grow best when free oxygen is available ; but most species can dispense with it and obtain O_2 from the compound substances in tissues or culture-media (facultative anaerobes).

A few species (obligatory anaerobes, e.g. *Clostridium*) cannot grow in the presence of free oxygen ; others can only tolerate a little (microaerophilic species).

Special Growth Requirements. A considerable number of pathogenic bacteria require something more than is present in the common culture media made with meat-infusion and peptone. The genus *Haemophilus*, for instance, must have blood or some substitute (fresh potato will do in some cases) ; and *Myc. tuberculosis*, when fresh from the body, will seldom grow in plain meat-infusion media, but will do so if glycerin is added. This has doubtless something to do with the large proportion of lipoids in this microbe. Generally speaking, pathogenic bacteria adapt themselves to a large degree to the saprophytic life of the laboratory by relinquishing their fastidious habits ; and, in doing so, they lose either temporarily or even permanently their pathogenic power.

A number of pathogenic cocci (*Pneumococcus*, *Streptococcus haemolyticus*, *Meningococcus*) need an accessory growth-factor not present in synthetic media. There is usually a little in the ordinary meat-extracts, and it appears to be present in fruit-juices and various other animal and vegetable extracts. To obtain full growth of the bacteria mentioned and also of many other species, blood-serum is usually added to the medium. The relation of the growth-promoting factor to the vitamins necessary for the adequate diet of animals has not been worked out, but the available evidence suggests similarity without identity.

Temperature. The temperature at which bacteria

will multiply in a nutrient medium ranges from 4° C. to about 70° C., but the vast majority of species thrive best at either 35° to 39° or 20° to 25°.

The few that prefer cold are termed psychrophilic, and are evidently species adapted to Arctic conditions. At the opposite end of the scale stands the scanty thermophilic group, which is to be found in the water of hot springs.

Between these are two great groups, one of which lives in the outer world, in water or soil or infests plants and cold-blooded animals, and the other is parasitic on warm-blooded creatures.

Other Physical and Chemical Influences. Among the many agents that affect the activity of bacteria we may mention light, hydrogen-ion concentration, salt-action and ' antiseptic ' substances.

No bacteria need light, and all are killed by sufficient exposure to it. Direct sunlight will sterilize a thin film of bacteria in a few hours, the time varying in inverse proportion to the intensity of the light. Spores resist a good deal longer, but succumb in a few days.

It is, of course, the chemical, ultraviolet rays that act most strongly, though certain visible wave-lengths are fatal if the cells are first sensitized with certain fluorescent dyes.

The Hydrogen-ion concentration in which bacteria in general thrive best is in the neighbourhood of pH 7·0. Concentrations above 6·0 and below 9·0 are usually unsuitable for growth ; and any greater divergence from neutrality, particularly on the acid side, is extremely harmful.

Salt-action. Bacteria are far less sensitive to variations of osmotic pressure than other unicellular organisms. In distilled water they can often survive for long periods, though very sensitive to slight impurities, such

as the minute traces of metal (1 in some millions) derived from a copper still. In tap-water the survival is much longer and some multiplication may take place. Small amounts of non-poisonous salts stimulate metabolism and growth. High concentrations of NcCl are well-tolerated, but growth is inhibited by the dehydrating action of the salt on the nutrient proteins of the medium ; and the dissociated ions have some bactericidal power. Preservation of meat, etc., by salting is almost entirely due to the inhibition of bacterial growth by dehydration.

Growth and Reproduction

Bacteria normally multiply by simple binary fission. First the cell enlarges to nearly double its original length ; a constriction then appears near the middle, or a septal line begins to be seen, and soon the two halves separate by the splitting of the new formed septum.

The various genera differ greatly in their ' post-fission movements ' as Graham-Smith termed them, and the microscopic and visible characters of their colonies depend on the arrangement of the cells as well as on their structure (Fig. 4).

The course of development of any freely growing bacterium in a typical ' culture ', such as a tube containing 5 or 10 c.c.m. of nutrient meat-broth, at the best temperature (usually about 37°C.) is shown graphically in Fig. 5.

A culture is made by introducing a tiny quantity of material containing bacteria into the new medium, which is then put in the incubator at the proper temperature. If the organisms are in the resting condition there is a delay of some two hours before obvious multiplication sets in, though a slow growth of the cells really starts much sooner. This is called the ' lag-phase ' (a-b). It does not occur when the organisms

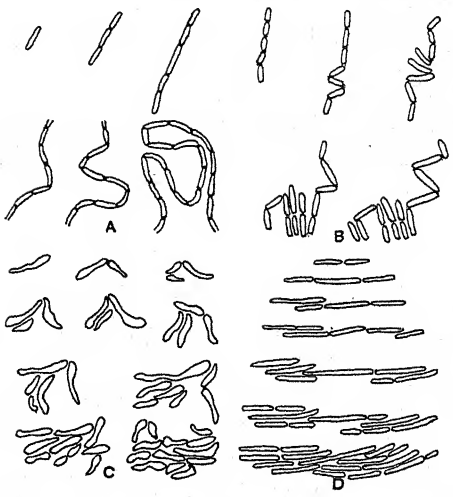

FIG. 4.—The post-fission movements of Bacteria. (After Graham-Smith)

A, Hoop-forming (Bac. anthracis). *B*, Folding (Past. pestis). *C*, Snapping (Cor. diphtheriae). *D*, Slipping (Bact. typhosum).

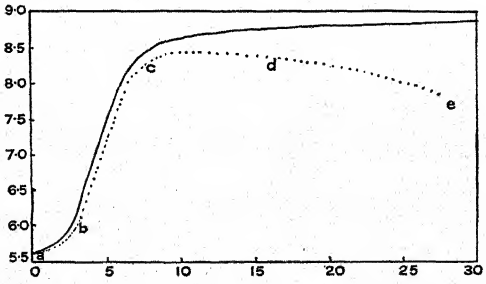

FIG. 5.—Multiplication of Bacteria (after Topley and Wilson)

Total cells——; living cells.....; *a b* = lag-phase; *b c* = logarithmic phase; *c d* = stationary phase; *d e* = phase of decline.
Ordinates = log. of number of cells.
Abscissae = time in hours.

introduced are already in a state of rapid multiplication. The lag-phase now changes quickly into the phase of maximum growth, which we call the 'logarithmic phase' (b-c) on account of the approximately straight line given by the graph of the logarithms of the number of cells in the culture plotted against the time.

The graph shows that from the first a certain proportion (calculated at about 20 per cent) of the cells newly generated either die or at least are incapable of regeneration when planted on fresh medium. This proportion increases during the next period, which is termed the stationary phase (c-d) since the living population is either at a standstill or slightly on the wane, though some slow multiplication of the most active cells is doubtless still in progress.

Finally, there is a phase of decline, which progresses at a varying rate in different cultures or species, and terminates at length in extinction. Even here, multiplication continues, but is overbalanced by the death-rate.

The cause of the retardation after the logarithmic phase is not exhaustion of the food-supply, for if the culture is boiled and filtered through porcelain, the fluid will support the growth of a new culture. In all probability lack of oxygen is an important factor, and there must also be an accumulation of excretory products, some of which are volatile or unstable, that inhibit growth and damage the weaker cells.

Colony-formation. On the surface of a solid medium a cell or group of cells will grow into a more or less circular aggregate, the characters of which vary in the different genera and species, ranging from the tiny, transparent dew-drop colonies of a pathogenic species, such as *Haemophilus influenzae*, to the large, dry, tenaceous, opaque crinkled colonies of free-living organ-

isms like *Bacillus subtilis*. After a certain time the colony ceases to spread, apparently because its own excreta diffusing into the surrounding medium inhibit further growth. In a few motile species, e.g. *Proteus vulgaris*, the spread is so rapid on a moist surface that a confluent film is formed in place of colonies.

Although the appearance of colonies frequently affords us a rough differentiation of genera or species, yet not only do innumerable species produce indistinguishable colonies, but there is also a fairly wide variation in the colonies of a single species.

Alleged Life-cycle (cyclogeny) of Bacteria. Ever since the birth of Bacteriology there have been some who believe that the reproductive process of bacteria is more complex than simple fission. A complex life-cycle has been postulated, comprising a succession of forms, such as minute gonidia, often small enough to pass through porcelain filters ; an amorphous or ' symplastic ' phase ; a phase of sexual conjugation, and so on (Löhnis 1921 ; Enderlein 1925). The evidence is summed up by Thornton in Vol. I of the *System of Bacteriology* (1930), more favourably, in the writer's opinion, than it deserves. In the extensive literature of the subject one searches in vain for trustworthy observations of the transformation of phases ; and when one attempts to observe it directly, the objects alleged to be ' gonidia ', symplasms and the like show no signs of life, and can be explained with greater probability as lifeless products of disintegrating cells, or temporary cell-modifications without any developmental significance.

The most important recent revival of the theory that bacteria have an ultramicroscopic phase is due to French workers (Fontès, Vaudremer, Calmette, etc.) who claim to have proved it for *Myc. tuberculosis*, and have received much support from various quarters. But in

England, Germany and America, attempts to verify this work have been almost uniformly unsuccessful.

Whenever an experimental method includes filtration through porcelain or other filters, a certain amount of uncontrollable error enters (see Part II, p. 40). When prolonged observation of cultures that have been exposed once or more to the air is involved, a proportion of slow-growing contaminations is almost inevitable. The claim of d'Herelle and Hauduroy (see p. 101) that the bacteriophage may transform bacteria into an ultramicrobic phase is invalidated by these considerations —the technique is too unreliable to prove the thesis.

The only fully established divergence from the normal reproductive process of binary fission is the 'three-point multiplication' which we shall describe below.

Death of Bacteria. We have just seen that the viability of new-born bacteria in a good culture-medium is variable. Their individual resistance to the harmful effects of chemical and physical agents is equally so. If a disinfectant chemical substance is allowed to act on a bacterial population and the death-rate measured at intervals, a sigmoid curve is often obtained, which shows that the cells vary greatly in their resisting powers. In a typical case the distribution of this variable quantity could be expressed by a chance-distribution curve such as that in Fig. 6. The stronger the dose of poison the less evident is the first bend of the sigmoid curve, until with very powerful doses the curve takes a 'logarithmic' form, which means that the number killed at any moment is simply proportional to the living population the moment before. All this falls in well with what we know of the type of distribution of biological variables in general and with various experimental data, such as the rate of haemolysis of suspensions of erythrocytes.

As we shall see later, the recognition of the mode of

distribution of variable characters in a population is of the greatest importance for the understanding of the bacteriophage phenomenon.

Classification of Bacteria

This is in a very unsatisfactory state, since our criteria for establishing species, genera, etc., are so imperfect. In many groups the different species look exactly the same,

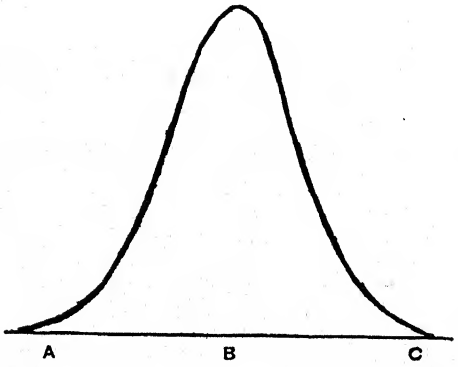

Fig. 6.—Theoretical Distribution of a variable character in a Bacterial Population

A, Above the average. *B*, Average. *C*, Below the average. Ordinates = the percentage of the population.

and have to be distinguished by their biochemical functions and by their reactions with the specific antibodies, especially 'agglutinins' which appear in the blood-serum of animals injected with living or dead bacteria. These properties are often variable, and can only be used as specific characters if their stability and range of variation have been adequately established over a considerable period.

Nevertheless there is no difficulty in establishing broad groups, which may be called Genera, though it

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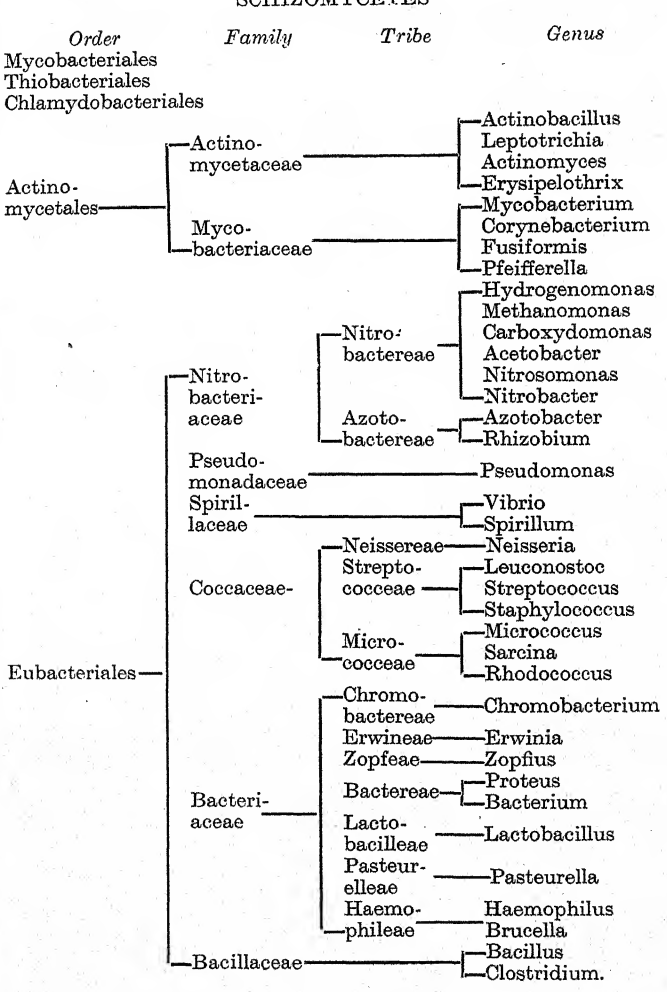

is not infrequently uncertain in which genus to place a particular species.

The nomenclature has until recently been chaotic, but a committee of American bacteriologists have lately devised a classification which, though not yet universally accepted, is receiving steadily increasing support. This system, slightly modified in accordance with Topley and Wilson (1929), will be used throughout this treatise (see page 17).

Abridged Specification of the Genera

Actinobacillus. Small chained clubbed, or filamentous unbranched rods. Gram-negative, sporeless, non-motile. Parasitic.

Leptotrichia. Thick long unbranched threads, clubbed or tapering. Usually anaerobic. Non-motile, sporeless, gram-positive. Usually parasitic.

Actinomyces. Branched mycelium, segmenting into short rods. Gram-positive. Aerobic, non-motile, sporeless. Forms masses with radially striated periphery. Parasitic.

Erysipelothrix. Rods, often long and branched. Non-motile, sporeless, gram-positive. Micro-aerophilic. Usually parasitic.

Mycobacterium. Thin gram-positive, acid-fast, sporeless, non-motile, aerobic rods; slow growth. Some pathogenic to animals and man.

Type: *Myc. tuberculosis* (the tubercle bacillus).

Corynebacterium. Gram-positive, sporeless non-motile, aerobic rods, often clubbed and banded. Some pathogenic to animals and man.

Type: *C. diphtheriae* (the diphtheria bacillus).

Fusiformis. Gram-variable, fusiform, anaerobic, sporeless non-motile rods. Feeble growth in all media. Some pathogenic to animals and man.

Type : *Fusiformis termitidis* (Bacillus fusiformis).

Pfeifferella. Small gram-negative sporeless, non-motile aerobic rods. On potato, brown honey-like film. Pathogenic to animals and man.

Type : *Pf. mallei* (the glanders bacillus).

Hydrogenomosas, *Methanomonas*, *Carboxydomonas* — Short motile rods that obtain energy by the oxidation respectively of Hydrogen, methane and carbon monoxide. Non-pathogenic.

Acetobacter. Chain-forming, non-sporing, non-motile aerobic rods. Oxidize alcohol and acetic acid, non-pathogenic.

Nitrosomonas. Motile or non-motile non-sporing aerobic rods or spheres. Oxidize ammonia to nitrites; non-pathogenic.

Nitrobacter. Non-motile, sporeless, aerobic rods. Oxidize nitrites to nitrates, non-pathogenic.

Azotobacter. Large motile or non-motile aerobic, sporeless rods and spheroids. Oxidize carbohydrates. Can fix atmospheric nitrogen. Non-pathogenic.

Rhizobium. Small rods and irregular forms. Motile, sporeless aerobic. Fix atm. N; Produce root nodules in Leguminosae. Non-pathogenic.

Type : *Rh. leguminosarum.*

Pseudomonas. Aerobic sporeless rods, generally gram-negative and motile. Produce soluble pigment; some pathogenic to animals or plants.

Type : *Ps. pyocyanea* (Bacillus pyocyaneus).

Vibrio. Curved, aerobic, sporeless, motile, generally gram-negative rods. A few pathogenic to man.

Type : *Vib. cholerae.*

Spirillum. Aerobic spiral rods, generally gram-positive and motile, sporeless, sometimes pigmented; non-pathogenic.

Neisseria. Small aerobic, non-motile, sporeless, paired spheres (cocci). Delicate growers. Mostly pathogenic to man and animals.

Type : *N. gonorrhoeae* (the gonococcus).

Leuconostoc. Paired or chained, non-motile, sporeless spheroids with gelatinous envelope ; usually gram-positive ; non-pathogenic.

Streptococcus. Chained, non-motile sporeless spheroids, sometimes capsulated. Mostly gram-positive and aerobic. Many are pathogenic to man and animals.

Type : *Str. haemolyticus.*

Staphylococcus. Clustered, non-motile, aerobic, gram-positive, sporeless spheroids. White or yellow. Mostly pathogenic to man and animals.

Type : *Staph. aureus.*

Micrococcus. Spheroids arranged in pairs and tetrads. Aerobic, sporeless, non-motile, generally gram-positive, usually non-pathogenic.

Sarcina. Similar to micrococcus, but forms cubical packets.

Rhodococcus. Spheroids in groups or packets. Aerobic, non-motile, sporeless, usually gram-positive. Form red pigment. Non-pathogenic.

Chromobacterium. Small, aerobic, sporeless rods, usually motile and gram-negative. Form yellow, red or violet pigment. Non-pathogenic.

Erwinia. A miscellaneous group of organisms pathogenic to plants. Mostly rods, non-sporing and aerobic with whitish slimy growth.

Zopfius. Long, chained, gram-positive, non-sporing, aerobic, motile rods ; non-pathogenic.

Proteus. Irregular, gram-negative, aerobic, sporeless, motile rods ; spreading growth. Liquefy proteins ; slightly pathogenic to man and animals.

Type : *Pr. vulgaris.*

Bacterium. Aerobic, sporeless, gram-negative rods; mostly motile, some capsulated. Ready growers. Mostly intestinal parasites; many pathogenic.

Type: *Bact. coli.*

Lactobacillus. Non-motile, sporeless, gram-positive rods, mostly aerobic. Produce (usually) lactic acid from carbohydrates; non-pathogenic.

Pasteurella. Small gram-negative, aerobic, sporeless, non-motile, ovoid rods; stain deeply at the poles. Pathogenic to man and animals.

Type: *Past. aviseptica.*

Past. pestis = the plague bacillus.

Haemophilus. Very small, often spheroidal rods. Aerobic, non-motile, sporeless, gram-negative. Need blood or a suitable substitute for growth.

Type: *H. influenzae.*

Brucella. Small, sporeless, non-motile, gram-negative, rods and ovoids; aerobic or needing CO_2. Pathogenic to animals and man.

Type: *Br. melitensis.*

Bacillus. Aerobic, sporing rods, mostly gram-positive. Spores hardly wider than cell. Few pathogenic.

Type: *Bac. subtilis.*

Clostridium. Sporing rods, partially or completely anaerobic. Spores wider than cell. Generally gram-positive; often motile. Many pathogenic to man and animals.

Type: *Clost. butyricum.*

§ 2. VARIATION OF BACTERIA

The word variation is used in the widest sense to include all divergences of individuals and populations from the accepted mean. The absence in bacteria of even the most primitive sexual mechanism makes it hazardous to apply to them the accepted terminology of

genetics. Even such terms as Reproduction, Heredity and Generation take a different shade of meaning when applied to organisms whose multiplication is as much akin to somatic growth as to germinal reproduction. Again, we should not apply the terms Modification and Mutation without a clear realization that there is in bacteria no obvious distinction between soma and germplasm. Even if we infer the existence of genes, and attribute racial variations to alterations of one or more of them, we are left with the fact that the general protoplasm is also handed on to the progeny, and therefore must carry with it all its characters, whether they are from the racial point of view permanent or temporary.

Owing to the minuteness of the cells it is impossible in most cases to determine the qualities of individuals, and for this reason the usual subject of study is a ' population ' or a race. It follows that most characters have already been transmitted to the offspring for many generations before they can be observed. Our usual problem, therefore, does not so much concern the transmissibility of the character to the immediate progeny of a cell as its degree of racial fixity.

Variation of the Reproductive Process

In spite of constant attacks, the classical doctrine that bacteria, with the exception of some species of Actinomycetaceae, multiply by simple binary fission has always succeeded in holding its main position. In recent years there has been a renewal of these attacks by a number of workers who believe themselves to have established a complex life-cycle for bacteria—a cycle that involves a variety of successive forms, such as a shapeless ' symplasm ', apparently formed by the fusion of a number of cells, and an invisible ultramicroscopic phase. Sexual conjugation has also been inferred from

the appearances in dried and stained films. It is impossible to review this work critically in the space at our disposal and the reader must be referred to Löhnis's (1921) monograph and to the article by H. G. Thornton in the *System of Bacteriology* (1930). In our opinion the evidence advanced and accepted by the supporters of this theory is not of a quality to bear the weight of the construction they put upon it.

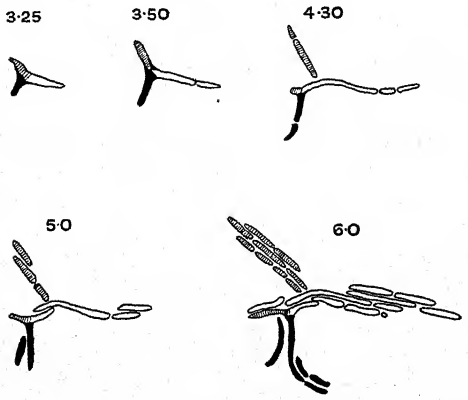

FIG. 7.—Three-point multiplication of *B. dysenteriae* (Shiga). Drawn with Zeiss-Abbé apparatus at the time-intervals shown. The bacilli originating from the three points are indicated by different shading

There is, however, a proved exception to the rule of binary fission. It has been shown that most, if not all, species of rod-shaped bacteria produce, in certain circumstances, branched cells, or 'Y-forms'.

For a long time cells of this kind, which had been seen in stained preparations of *Corynebact. diphtheriae* and *Myc. tuberculosis*, were held to be merely 'involution forms', that is, degenerate and dying cells. But in

1915 Hort succeeded in observing directly the growth and multiplication of Y-forms in *Bact. typhosum*, and showed that new, living cells were produced by all three branches of the Y. Later, in 1921, the writer re-discovered this phenomenon (without knowledge of Hort's work, which had attracted little attention), and proved

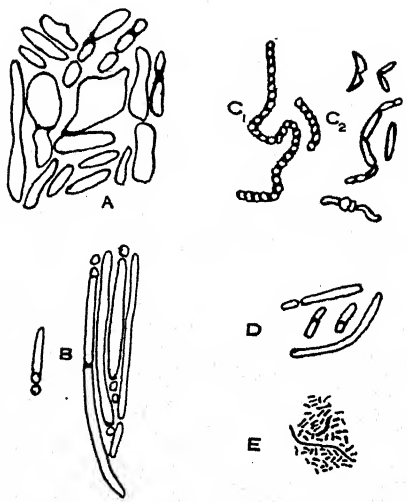

FIG. 8.—Irregular Cell-division

A, Bact. coli. *B*, Vib. cholerae. C_1, Streptococcus (normal). C_2, Streptococcus (irregular). *D*, Bact. typhosum. *E*, Haem. influenzae.

that it occurs regularly in a large number of species when an old and largely dead culture is revived by planting in fresh nutrient culture-medium (Fig. 7). It is usually accompanied by the production of odd-shaped cells and bizarre, swollen forms, many of which burst and dissolve after one or two fissions (see Fig. 8). The term 'three-point multiplication' was coined for this

atypical form of division, and it was viewed as a peculiar reaction to the injury caused by sojourn in an increasingly harmful environment. The progeny of single Y-forms of *Vib. cholerae* proved to be perfectly normal on the two occasions when the difficult technique of isolation was successfully carried out.

Cellular and Racial Modification

There is a physiological fluctuation in the average size of the cells in a culture at different periods of its growth. During the lag and logarithmic phases, the average length and width are greatly above those of the cells of the mature culture.

There is also a fairly wide variation of mature individuals, affecting characters such as length, intensity of staining, and resistance to physical and chemical agents. Variation of this kind occurs spontaneously in ordinary cultures, and can be much intensified by cultivation on media containing low concentrations of antiseptic substances, such as dyes, or by maintaining the culture in an almost exhausted fluid medium. By raising sub-races from exceptionally long individual cells of *Bact. coli*, Barber showed that the durability of the change varies between the extremes of immediate reversion and apparently complete fixity. One of the variant races was observed for three years without showing any signs of reversion. We may accept this racial modification as equivalent to mutation, though we have no means of distinguishing it clearly from cellular modification.

Some races of streptococci produce cells of very irregular form and size, so that it may at first sight be difficult to distinguish them from rods (e.g. *Streptococcus mutans*. Fig. 8 C_2). In the same Figure we show (A) a minute colony of a race of *Bact. coli* whose cells grew

in fantastic shapes, many bursting and dissolving after a period of growth. At B is seen the production of 'gemmules' in *Vib. cholerae*. These do not survive or reproduce when planted on a fresh medium, and they therefore appear to be abortive cells. The so-called involution forms of bacteria are either morphological variants usually of lowered vitality, or normal cells swollen and distorted by physical or chemical injury.

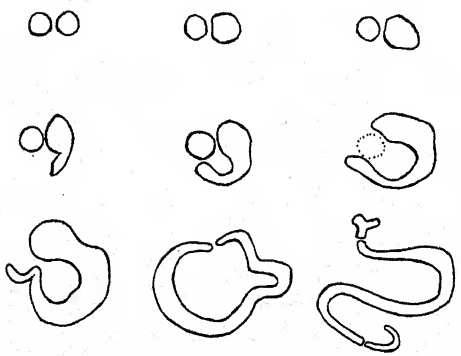

FIG. 9.—Development of abnormal and branched forms from a 'coccoid' cell of an old culture of Vib. cholerae when put into fresh medium. Drawn at intervals during 48 hours. The left-hand coccoid died and disappeared by autolysis in 24 hours

In the latter category are the spherical forms, many of which are motile, that may be seen in almost exhausted cultures of *Vib. cholerae*. When transferred to fresh medium some dissolve, and others develop into irregular forms with a tendency to branching (Fig. 9). *Azotobacter radicicola* grows in the root nodules of the Leguminosae in the form of irregular 'bacteroids', some of which are Y-shaped. These modifications are usually transitory, but it is possible to raise races of bacteria (e.g.

Bact. coli) which retain for very long periods the tendency to produce distorted, branching and autolyzing cells.

Recurrent and Alternating Variations

Variation of Biochemical Functions. The power of utilizing carbohydrates and similar substances for energy-production is, in general, sufficiently constant in a given species to provide a criterion for its differentiation from allied micro-organisms. But it has been found necessary to exercise great caution in the interpretation of any single series of fermentation-tests, for in some cases the biochemical functions of a race may undergo profound alterations during further cultivation. In the species-group of *Streptococci*, for instance, a classification worked out on these lines by Gordon was accepted for a time, until it was shown by Ainley-Walker that the properties of many races underwent such changes under prolonged cultivation, that a repetition of the earlier tests gave a totally different grouping of the same range of races.

The temporary suppression (often for a great number of 'generations') of one or more of the physiological properties characteristic of the species is a frequent source of worry to practical bacteriologists in their endeavours to identify newly isolated cultures. For example, *Bact. dysenteriae* (*Flexner*) when first isolated may refuse to ferment Mannite, though it invariably does so after a period of cultivation. Similarly we occasionally meet with modified races of *Bact. paratyphosum B.* which fail to produce gas from glucose. Most of these changes have been but casually recorded, and have not been studied with any thoroughness. Considerable attention, however, has been given to a curious phase-alternation, which was described by Neisser and later by Massini, in a species of *Bacterium coli* called

'mutabile' and which was subsequently observed in a number of species in the same and other genera. *Bact. coli mutabile* differs from the common *Bact. coli* in failing, on first isolation, to ferment lactose. Thus, if a small amount of a culture is spread on the surface of agar containing lactose and an indicator-dye the colonies that develop show at first no colour-change indicative of acid-production from the lactose. But after further incubation little knobs or papillae appear in the substance of the colony, enlarge, and gradually take the colour (e.g. red) indicating acid-production (Fig. 10).

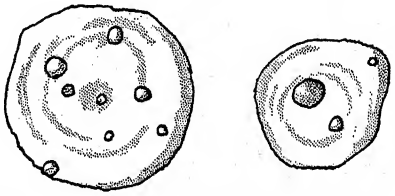

FIG. 10.—Colonies with Daughter-colonies

These knobs are called daughter-colonies, and represent the upspringing of modified races able to digest the lactose.

If a sample of cells from a red knob is spread on a fresh lactose-agar surface, some (or even all) of the resulting colonies will be red from the beginning, whereas a similar spreading from an uncoloured part of the colony gives only colourless colonies which, in their turn, will produce acid-forming papillae.

The lactose-fermenting sub-races, which are indistinguishable from the common *Bact. coli*, breed true under ordinary conditions, but can often be induced to revert either by cultivation on agar containing phenol or by passage through animals. Similar facts have been established in some races of *Bact. dysenteriae*.

In addition to lactose, certain other substances such as saccharose and dulcitol will induce a parallel variation in *Bact. coli mutabile*; the daughter-colonies in each case having the power to ferment the particular substance presented to them. These races also show their inherent variability by the production of colourless daughter-colonies in the absence of fermentable carbohydrate. The sub-races thus arising differ from the stock in some character such as morphology or capsule-formation.

The most probable explanation of this phenomenon is that in the mother-race the fermenting powers are suppressed as the result of parasitism, but can be re-developed in various directions under the 'stimulus' of appropriate fermentable substances.

An interesting attempt has been made by Stewart (1927) to bring these phenomena into the scheme of Mendelian genetics, on the assumption that either conjugation of cells or at least autogamy occurs during the formation of the papillae. The case, however, remains unproved, since the recorded phenomena do not correspond completely with Mendelian genetics, even on the qualitative side; and no quantitative data are given, because they were unobtainable. Further, the assumption of autogamy or conjugation is not supported by facts.

Moreover, the efficacy of a specific chemical stimulus can have no connexion with genetics. Indeed it suggests some quite different mechanism, such as a variable balance of antagonistic factors, which alternate in dominance as the changing environment offers now to one now to the other the opportunity of developing its characteristic function.

The experimental 'adaptation' of bacteria to a new diet can be explained in a similar way.

By cultivating a race of *Bact. typhosum* fortnightly for two years in a medium containing 2 per cent of lactose, which the species normally does not ferment, Twort succeeded in 'training' it to do so. A similar 'training' in the fermentation of dulcitol was achieved by Penfold with the same species. He showed that not all individuals acquired the new property, but that fermenting variants appeared and gradually supplanted the parent-type.

These variants were generally unstable and showed a strong tendency to reversion when grown on media without lactose; but if the cultivation in the carbohydrate medium was greatly prolonged, this tendency diminished, though no evidence of complete irreversibility was obtained in the period of observation.

If the lactose-fermenting variant races were true mutants, they would presumably not lose their newly acquired power so readily. It seems that in this species the factor inhibiting fermentation is much more fixedly dominant than in *Bact. coli mutabile*, and that the fermentation factor only achieves self-expression when the race is hard-pressed for other forms of nourishment, as it is in fortnight-old cultures. It is held by some authorities that the sugar does not incite the specific variation, but merely selects spontaneous variants. But since there is no evidence that either *Bact. typhosum* or *Bact. coli mutabile* produces lactose-fermenting variants in the absence of lactose, we are forced to admit the direct action of the sugar.

Smooth and Rough Variants. In a large number of species of the most diverse genera, after a varying period of cultivation in artificial media, the character of the growth tends to change.

If, for instance, a number of stock laboratory races of *Bact. typhosum* are analysed by plating (see Glossary),

the colonies of some of them will be of two strikingly different types. One, the normal, is circular, and has a moist, evenly glistening smooth surface; whereas the other is called Rough, because of its wavy, spreading margin, and crinkled, dry-looking surface (Fig. 11). Microscopic examination of the two types of colonies reveals no constant difference in the cells, though the

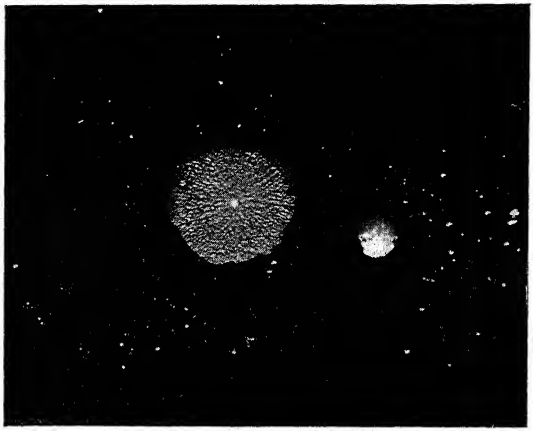

FIG. 11.—Rough and Smooth Colonies

more extreme Roughs tend to grow in long, segmented filaments. In fluid media the Smooth phase produces uniform turbidity, because the cells are evenly dispersed in the fluid; but the Rough phase grows in little clumps, which tend to fall to the bottom of the tube and leave the fluid more or less clear (Fig. 12). This is an expression of the sensitiveness of the cells to flocculation by the NaCl in the medium.

The Roughness of colonies is due to the adhesion of

the cells in irregular strands or bundles. Smooth cells, on the contrary, separate easily, and pack uniformly.

It has been shown that a change of chemical structure takes place in the change from Smooth to Rough. If suspensions of the two types are injected into separate rabbits, in due course the serum of the two animals will be found to have different properties; for the Smooth and Rough 'antigens' produce different antibodies, distinguishable by the agglutination-test.

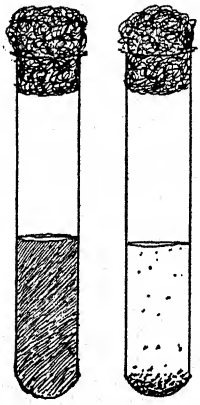

FIG. 12.—Smooth and Rough Phases of a Bacterium in a fluid medium

Thus we have an antigenic change underlying the alteration of the visible character of the cells and the acquisition of sensitiveness to electrolytes. But this is not all. A great loss of virulence or toxicity accompanies the antigenic change to the Rough phase.

In the genus *Bacterium* the Smooth phase has been shown by chemical methods to possess a particular protein-carbohydrate complex which is missing in the Rough state, and there is little doubt that the difference of the two phases in pathogenicity and antigenic character is due to this.

Although the Rough phase is occasionally found in slow and relatively mild infections in man and animals, it is much more common in the laboratory, and the longer a race has been under cultivation the more likely is it to produce the Rough variant. Many old stocks, in fact, turn completely Rough.

There can be no doubt that cultivation on artificial media induces the variation. Certain species, e.g. *Bact.*

dysenteriae (*Sonne*), invariably throw off R variants in the first or second culture after its isolation from the human body, and unless special precautions are taken to preserve the S-phase by selection, the whole race rapidly turns Rough.

A very interesting case is that of *Haemophilus pertussis* (the microbe of whooping-cough), which the writer has recently been investigating, with Mr. P. H. Leslie. In order to grow this organism from the respiratory tract of a whooping-cough subject, a special medium containing fresh blood must be used (hence the generic name, *Haemophilus*), but after a certain number of subcultures the microbe can usually be coaxed to grow in a plain meat-extract medium.

Long before the recognition of the S-R variation, the discoverer of *Haem. pertussis*, Jules Bordet, observed that this acquisition of the faculty of growth on simple media is accompanied by the very two changes we have just described: loss of toxicity to animals, and a profound antigenic alteration. We have found, in addition, that the colonies are on an average more granular and uneven, and that an irregular increase of sensitiveness to electrolytes (clumping) can sometimes be detected. For these and other reasons we regard it as a case of the S-R. phase-variation, although the Roughness and salt-sensitiveness are less striking than the other features of the change.

Roughness is itself variable. In the same culture one may see colonies of several grades intermediate between Smooth and typical Rough. The permanence of the change also varies from easy reversibility to apparently complete fixity.

This type of variation has been observed in the following genera: *Bacterium*; *Mycobacterium*; *Corynebacterium*; *Proteus*; *Pasteurella*; *Streptococcus*; *Bacillus*; *Clostri-*

dium. It is therefore a widespread, and possibly universal phenomenon in pathogenic bacteria, but its character and frequence vary greatly in different species.

It can be interpreted as the adaptation of a parasitic organism to a saprophytic life. The Smooth antigen, bound up as it is with toxicity and virulence, is useful only in the former state, so that under cultivation the Rough variants, whether appearing spontaneously or, as seems to be the case, in response to chemical stimulation by artificial food, tend gradually to outgrow the Smooth.

The term Mutation does not seem properly applicable to a type of variation that can be induced with some constancy in a large number of species by definite nutritional stimuli and shows a high degree of reversibility. Like the variation of carbohydrate fermentation, which we have just described, it can be accounted for by the balance of two antagonistic factors which prevail alternatively in different sets of circumstances. The change from one to the other appears to be abrupt, but varies in completeness, since intermediate or half-Rough forms are common. The term Alternation, introduced by Toeniessen, gives the best indication of the nature of the process. We must, however, admit that its extreme effects may be indistinguishable from those of a true mutation, for permanent fixation of one of the Rough phase is not uncommon, especially when the effective stimulus is prolonged.

The Specific-Nonspecific Phase Alternation. Another very interesting type of Variation was first described by Andrews and elaborated by Bruce White and others, in the group of species of the genus *Bacterium* called *Salmonella* (after Salmon, its discoverer), in which the important bacteria of typhoid and paratyphoid fever and of food-poisoning are included. Here the chemical complex or antigen of which the flagella are mainly

composed, and which is quite different from the somatic antigens, has two alternative constitutions which are found separately in the different colonies of a pure-line culture. The one constitution is called Specific, since it is uniquely characteristic of the species (or perhaps more properly, variety), the other non-specific because

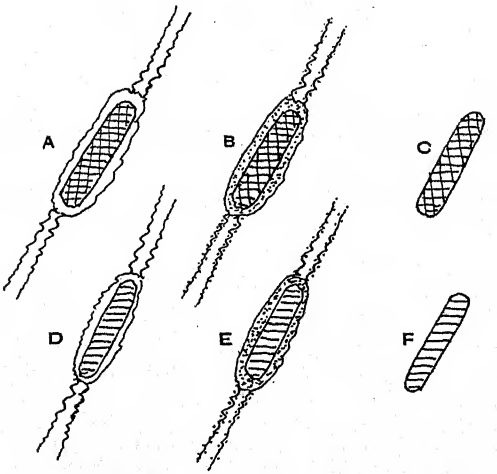

FIG. 13.—Phase-variation of a Diphasic Salmonella Bacterium
A, Smooth, specific, motile. *B*, Smooth, non-specific, motile. *C*, Smooth, non-motile. *D*, Rough, specific, motile. *E*, Rough, non-specific, motile. *F*, Rough, non-motile.

it is possessed in common by related species within the group, provided that they are also in the non-specific phase (Fig. 13). Species that show this phenomenon are called ' Diphasic '.

In platings of such a species, if a number of colonies are tested for agglutination with two antiserums, one (*a*) made with the same species, and the other (*b*) made with a different, but allied species, some of the colonies

will react only with serum (*a*), since it is the only one that contains the specific agglutinin, while others will react with both serums, because both contain the non-specific agglutinin for the group. We may represent the phenomenon schematically thus :

	Bacterium X.		Bacterium Y.	
	Specific Colonies.	Non-specific Colonies.	Specific Colonies.	Non-specific Colonies.
Antiserum against species X (mixed phases) . .	+	+	O	+
Antiserum against closely related species Y (mixed phases)	O	+	+	+

+ = Positive agglutination-reaction.
O = Negative agglutination-reaction.

The first sub-culture from a single colony usually reproduces the same phase-antigen in a fairly pure state, but the alternate phase generally reappears in a certain proportion of individuals at about the second transplantation.

There is, however, considerable irregularity in the phenomenon, and the prolonged persistence of a phase is not unknown. Permanent phase-fixation has not been observed under experimental conditions, but it almost certainly occurs in nature, for within the *Salmonella* group there are several permanently 'Monophasic' species which are identical with one of the two phases (usually the non-specific) of a diphasic species. It is evident that either the Monophasics have developed from the Diphasics by loss of a character, or the latter from the former by the acquisition of a new one ; and the first alternative seems the more probable.

The antigenic relationships of the members of this group are in reality even more complex than this, for while in some cases the non-specific components of different species are identical, in others they are similar but distinguishable.

So far as the specific-phase antigens are concerned, there are some twenty distinct ones recorded up to date, and the future will doubtless reveal more. This antigen is often the sole known distinctive character of the species, though in some cases it is supported by a difference in carbohydrate fermentation reactions.

The phenomena point to an alternation of the dominance of two finely balanced factors, somewhat similar to the mechanism we have postulated for the Rough-Smooth variation. But in the present case we have no knowledge of an exciting stimulus nor any inkling of the significance of the change. It is clearly a possible means for the production of permanent varieties.

Variation of Motility. Another type of alternating variation that occurs in the genus *Bacterium* as well as in certain other genera containing motile species (e.g. *Proteus*), concerns the presence or absence of flagella, and consequently of motility. Here, as usual, we are dealing with the character of races and sub-races rather than of individuals. A race is considered motile if most of its cells show motility in young fluid cultures.

The phenomenon is most striking in *Proteus*, which normally produces two kinds of growth on solid media ; one of which takes the form of ordinary circular smooth colonies, and the other spreads rapidly in a thin film all over the surface of the medium. The latter owes its spreading power to an exceptionally active motility ; the former is constituted of non-motile cells devoid of flagella.

In the *Salmonella* group an identical variation occurs, without any such striking difference in the type of

growth. Thus, non-motile variants have been isolated from races of *Bact. typhosum*, and *Salmonella aertrycke*, and have preserved their character for several years, i.e. many thousands of 'generations', though the writer has obtained a reversion of one of these (*Bact. typhosum*) to the motile form by incubating a meat-broth culture for three months. The change therefore seems to be more a suppression than a loss of the character.

It is easy to produce a fugitive suppression of flagella by cultivation at a temperature of 42° or 43° C., or in a medium containing a low concentration of phenol, but reversion takes place immediately on restoring the optimum conditions for growth.

In the *Salmonella* group, since the diphasic antigen resides in the flagella, and perhaps also in an ectoplasmic layer present only in flagellated cells, the non-motile individuals or races are not subject to the specific-non-specific phase-variation. The antibody (agglutinin) produced by injecting animals with the antigen of non-motile variants has no affinity for the flagellar antigens, and these latter are also distinguished from the somatic by their destructibility by heat (100° C.) and ethyl alcohol.

If a non-motile variant turns Rough under cultivation, (Fig. 13 F.) the resulting race can be regarded as a highly reduced form of the species, for not only does the flagellar layer, with its diphasic antigen, fail to develop, but the soluble specific substance characteristic of the Smooth state is also missing.

Other Types of Variation

The following characters have been found subject to variation of some degree of permanency; spore-formation; pigment production; range of carbohydrate-fermentation, capsule-formation; production of mucoid substance, oxygen and CO_2 requirements. An account of

these variations with a good bibliography is to be found in Arkwright's (1930) article. There can be little doubt that permanent variants in respect to these characters, are formed from time to time by Mutation but that the great majority are eliminated by natural processes.

Transmutation of Species. Bacteriological literature contains numerous reports of transmutation, that is of species producing variants identical with some other species. Of these reports we can only say that the evidence they present is by no means sufficient, either in quantity or quality, to prove the reality of the alleged phenomena. No such occurrence has ever been observed and confirmed under fully controlled experimental conditions.

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PART II

THE VIRUSES

§ 1. GENERAL CHARACTER AND PROPERTIES

IN many diseases of human beings, animals and plants (see page 67) no bacteria can be incriminated; yet in their obvious infectivity and in the general course of their symptoms these diseases run so clearly parallel with the bacterial infections, that we cannot help assuming for them a living cause analogous to bacteria. Creatures of sub-microscopic dimensions are therefore imagined to lodge in the tissues and fluids of the infected organism, and there to give rise to symptoms of disease, by deranging the normal functions of the cells.

The best definition of a virus that the present state of our knowledge allows is: 'An infective agent below the size-limit of microscopic resolvability.'

In this we intentionally avoid the words 'filterable' and 'invisible', which are often used in defining viruses, since, as we shall see, both are open to question. The name virus is itself unsatisfactory, since it is undefinitive, and is sometimes used to include bacteria. 'Ultra-microbes', in spite of its hybrid origin, has the advantage of signifying a minuteness beyond the powers of the microscope to define; nor has it ever been used in any other sense.

Filterability. A general, though rather inconstant, property of viruses is that of passing through porcelain and other filters which retain all ordinary bacteria.

Although it is clear enough that any filter will remove from a fluid all particles larger than the largest pore of the filter, common sense leads us astray over particles small enough to pass through, for in reality they do not necessarily do so. The behaviour of very minute particles is no longer governed by coarse mechanical principles but by the finer physical and chemical laws of adsorption and electrical charge. Thus the filterability of particles, in themselves small enough to pass the filter-pores, depends on such factors as the Hydrogen-ion concentration of the fluid and the electric charges both on the particles to be filtered and on the substance of the filter. Protein aggregates in solutions more acid than the isoelectric point of the protein will be adsorbed and will combine insolubly with the silicate anions of porcelain filters, and so be retained. If, however, the solution be alkalized the protein will pass through the filter. The same particle may be retained by one filter but pass through another of equal porosity but of different constitution and opposite electrical charge.

Cataphoresis experiments on the electric charge of viruses have given rather discordant results. Thus the vaccinia virus has been found to bear a negative charge in fluid of *p*H from 5·5 to 8·4. On the other hand, the foot-and-mouth virus is positively charged when the *p*H is anything up to 8·0. English workers have found that under the best experimental conditions both these viruses travel towards the anode, and the same has been recently demonstrated in America for that of poliomyelitis. We must, however, make the reservation that in all these cases it may be the particles on which the virus is adsorbed whose movements are being determined, rather than those of the virus itself. It has indeed been shown that when conditions are favourable viruses readily undergo adsorption on all

manner of particles. Further, the passage through filters is greatly influenced by temperature and pressure, and by the constitution of the fluid containing the virus, e.g. by the presence in it of colloidal particles capable of adsorbing the virus. Thus the herpes and vaccinia viruses will pass through filters when suspended in broth, but usually fail to pass when in salt-solution. It is very difficult to control and standardize these conditions so as to make one experiment comparable with another, and the most careful workers obtain puzzlingly inconsistent results.

Even if this were not so, filterability could not be used as a criterion for a virus, since certain visible organisms, such as spirochaetes, *Bact. pneumosintes* and the organism of bovine pleuro-pneumonia (see page 46) pass through filters that retain all ordinary bacteria.

Lippschütz states that the densest porcelain filters (e.g. Kitasato's) will not let through viruses, whereas the smaller particles in colloidal solutions pass readily. Further, it has been found that agar-gel membranes completely retain the viruses of vaccinia and fowl-pox.

Ultra-filtration. Collodion membranes of varying pore-size have been used lately to determine the approximate size of filtrable particles, such as viruses. The size of the pores depends chiefly on the strength of the collodion solution with which the thimble-shaped membranes are made, and it can be measured by estimating the pressure required to force water through, or by determining whether various mineral particles of known magnitude will go through. The size of viruses estimated by this and other methods is given in Table I.

How far it is possible to rely on the measurement of the pores either of collodion or of porcelain filters it is hard to say. But there is fairly good evidence that a channel must be several times greater than the smallest

diameter of a granule or rod before the latter will pass through under moderate pressure. The average pore-size of porcelain filters (Berkefeld, V, N and W; Mandler; Chamberland L3) seems to be from 2 to 10 μ, whereas the average width of ordinary bacteria and cocci is 0·5 to 0·75 μ. Nevertheless the organisms will not pass through (for further information see Rivers, 1928).

TABLE I

Various estimates of the approximate size of viruses and certain other particles.

(1 millimetre = 1,000μ = 1,000,000 mμ.)

Hydrogen molecule	0·16 mμ
Starch ,,	0·5 ,,
Saccharose ,,	0·7 ,,
Albumin ,,	4 to 10 mμ
Gold Sols.	6 to 95 ,,
Bacteriophage	8 to 30 ,,
Tobacco mosaic virus	30 mμ
Colloidal particles in a fresh 1 per cent Haemoglobin solution	30 ,,
Herpes virus	20 to 100 mμ
Foot-and-mouth disease virus	20 to 100 ,,
Mouse sarcoma virus	75 mμ
Viruses in general	75 ,,
Strongyloplasmata (viruses)	100 to 250 mμ
Bacterium pneumosintes	100 to 300 ,,
Rickettsia prowazeki	200 to 500 ,,
Wave-lengths of visible light	400 to 700 ,,
Chromobacterium prodigiosum	500 to 1000 ,,

Microscopic Visibility. By ordinary transmitted light particles of 200 mμ (0·2 μ) or perhaps less may be seen, but they are visible only by their diffraction-images, and it is impossible to see their shape, or to 'resolve' the combined image of two adjacent particles into its separate parts. Objects must exceed 250 mμ if their images are to be resolvable. Apart from size, the

object must have a refractive index differing considerably from that of the surrounding fluid, or it will remain invisible.

In dark-ground illumination the particles are very obliquely illuminated by means of a special substage condenser. No light passes direct from the condenser into the lens, and all that one sees is diffracted. Very minute particles, perhaps down to 10 or 5 mμ, may thus be seen as minute light dots on a black background, but the limit of resolution is the same as for ordinary illumination.

The resolving power of any lens depends entirely on the numerical aperture of the lens and on the wave-length of the light used, and it can be calculated by dividing half the wave-length by the numerical aperture. With invisible light of short wave-length the images can be photographed, and objects as small as 75 mμ can be detected and resolved; nor is this the possible limit, if technique can be improved. Thus if some viruses are, as Table I suggests, of the order of 75 mμ, they may have been seen by dark ground illumination, and photographically resolved by means of ultra-violet light. On the other hand, there is no certain means of identifying such granules with the virus, or of distinguishing them from the colloidal particles inevitably swarming in organic fluids.

Lippschütz believes that viruses, which he calls generically Strongyloplasmata, are microscopically visible and indeed to a large degree recognizable. He believes them to be only just below the limit of microscopic resolution, and maintains that they can be stained by Giemsa's method or by Loeffler's flagella-mordant. They are seen in dried fixed films thus stained as minute round, sharply contoured dots, often in great numbers and massed together in clumps or lying separate and

sometimes showing double-forms which may represent fission. Strong support is given to this view by some English authorities who believe that the tiny bodies visible in vaccine-lymph and first described by Paschen in 1906 are actually the virus. They can be observed in the tissues after injection, and appear to multiply and be ingested by phagocytes, just like cocci and other bacteria. A recent demonstration of these bodies by Ledingham and others has convinced the writer that they are in truth microbes. The staining process seems to enlarge them and bring them just above the limit of resolvability, so that a constant form is perceptible. The large cell-inclusion bodies have been shown to consist of innumerable massed ' elementary corpuscles ' of this kind.

The view that the viruses, or some of them, are visible, is however not yet generally accepted. To form the opinion that the granules that one sees are the virus is undoubtedly easier than to prove it.

The alternative theory, that we are dealing with soluble formless agents which may, or may not, be termed ' living ', as we please, has frequently been argued since the time of its first suggestion by Beijemick more than thirty years ago. It was resuscitated in a different form by Sanfelice, and introduced into Biochemistry by B. Moore, who envisaged a class of lifeless but transmissible autocatalysts, which are continually renewed by the cells on which they act. We shall see later that the phenomena of bacteriophagy have revived this theory, which had tended to drop out of orthodox considerations of the nature of viruses.

There is no doubt that we are naturally biased in favour of the solid or semi-solid nature of all living beings; if only because all those that we know have form and solidity; and it must be admitted that nothing

has emerged from the study of viruses that compels us to abandon this prejudice. On the other hand, we need not feel committed to the view that all the 'viruses' are organisms. The name may well have been given to a heterogeneous collection of active agents, some of which are really very small microbes, and others are of an entirely different order of being. We shall return to this question later on.

Border-line Organisms. If there were a great gap between the sizes of bacteria and viruses there would be less reason to lean towards the living corpuscular nature of the latter. But in truth there is hardly any gap. One organism, indeed, has at one time been classed with the viruses, and at another with the bacteria. This is the infective agent of bovine pleuro-pneumonia, which resembles the bacteria in being just visible (resolvable) with ordinary illumination and cultivable on artificial media containing blood. Virus-like, it passes filters, though only those of coarser grade. Its average dimensions must be of the order of 250 mμ. Similar properties and dimensions are possessed by a group of minute bacteria known as *Bact. pneumosintes*, which have been cultivated from influenza in human beings, and were at one time held to be its cause. Having subsequently been found in many healthy throats, and being apparently absent in most epidemics of influenza, they are now regarded as harmless.

Slightly larger (up to 500 mμ or more) and unfilterable are a group of tiny organisms known as Rickettsia, after Ricketts, who first described them. They are generally held to be the cause of typhus (spotted) fever, Rocky-Mountain spotted fever, and the Transvaal cattle-disease known as 'heart-water' (from the pericardial effusion that commonly occurs). These bodies can be found easily in the excreta and certain intestinal cells

of lice, which carry the infection, and with more difficulty in the blood and tissues of infected animals or man. They stain very poorly with ordinary dyes, but show up with Giemsa's stain as purple dots or minute rods, sometimes surrounded by a paler halo. It is not possible to grow them on artificial culture media, but multiplication occurs when they are included in living tissue-cultures. Although it is possible to maintain that these objects are cell-inclusions due to a virus, and are not themselves bacteria, the great profusion in which they may be found in lice and the relative constancy of their form makes it much more probable that they are micro-organisms.

Centrifugalization. Various efforts have been made to prove the corpuscular nature of viruses by centrifugalizing and demonstrating a concentration of the active body in the depth of the centrifuge-tube. Two considerations, however, make the interpretation of such experiences very difficult. First, viruses are readily adsorbed on particles of many kinds and therefore must tend to be carried down with them on centrifugalization: and second, it is far from certain that particles of extreme minuteness would be appreciably moved by the centrifugal force available. In the case of the foot-and-mouth virus, spinning at about 4,000 revolutions per minute has been shown to effect no concentration.

§ 2. HABITAT. CONDITIONS OF MULTIPLICATION

Natural 'Life' of Viruses. There is no evidence that viruses occur anywhere except in living or recently dead organisms, or in fragments or particles detached from organisms; but it is, of course, not proved that such a thing is impossible. Though usually causing disease, viruses can persist, at least for a time, in apparently

healthy tissues, either after the organism has recovered from an attack of the disease or if it is a 'carrier' (infected but immune).

Seeing that pathogenicity is at present the only known positive character of viruses, non-pathogenic forms cannot be detected.

It appears that viruses live in cells, rather than in fluids. For instance, the viruses of rinderpest and fowl-plague are found in the blood and have been shown by centrifuging to be concentrated in the leucocytic layer. On the other hand, the virus of herpes has frequently been found on the surface of the human body, though there is no evidence that it multiplies there. It may also be present in apparently normal human saliva.

Resistance of Viruses to Physical and Chemical Agents. What little is known on this subject concerns heat, cold, desiccation and the actions of antiseptics such as phenol (carbolic acid), perchloride of mercury, alcohol, glycerin and a few others. There have also been some experiments with ultra-violet light, and radium. It is not necessary to discuss this subject in detail, as the resistance of viruses is, so far as has been determined, of the same order as that of the vegetative forms of bacteria and less than that of bacterial spores. They can live for years in a state of suspended animation. The poliomyelitis virus, for instance, has been kept without loss of virulence for eight years in fragments of spinal cord suspended in 50 per cent glycerin.

Conditions of Growth. In the presence of suitable living cells a virus lives and increases, but until very recently there has been no evidence that any growth can take place in the absence of living cells. Pure cultures of certain viruses such as vaccinia can be procured and maintained by injecting material into the testicles of rabbits and other animals where the virus

multiplies for a time, and then dies out. Again, when cells from suitable tissues are grown in tissue-culture (Carrel's method), the viruses of herpes and vaccinia can be cultivated with or in them.

The range of cells capable of supporting growth of any particular virus is often very narrow. For instance, many of the viruses of caterpillar-wilt can infect only one species of caterpillar. Again, the sarcomatous tumour of chickens described by Rous cannot be transmitted to any other species of bird[1] or animal. A similar specificity is found in the viruses of mammals; poliomyelitis (infantile paralysis), mumps and chicken-pox affect only human beings; several virus diseases appear to be confined to rabbits. Sometimes, it is true, a virus can attack more than one species; e.g. rabies (hydrophobia), which is readily transmissible from dog to man; but even in such cases there is a strong tissue-specificity, in that the central nervous system is the part involved in both species.

A considerable number of claims have lately been made of successful cultivation on lifeless media. One of the most promising has been concerned with the tobacco-mosaic virus, but confirmation has not been forthcoming. It was for a time believed that a just visible virus had been grown from human poliomyelitis, but recent opinion has turned against the identity of these 'globoid' bodies with the causative agent of the disease. Lastly we may mention the unconvincing reports of the cultivation of the Rous chicken-sarcoma virus and also of the alleged virus of disseminated sclerosis of the human central nervous system. The great difficulty of the technique involved makes experiments of this kind arduous and uncertain.

[1] Very recently a chicken tumour has been induced to 'take' in a duck. But it is an exception that proves the rule.

Some English workers, however, have recently reported that vaccinia virus will multiply in a liquid medium containing serum and the extractives from minced living tissue, with traces of debris from the cells. There appear to have been either no intact living cells at all, or so few as to be insignificant. It seems, then, that culture of some of the viruses in a lifeless medium may shortly become practicable.

Reproduction. Nothing whatever is known of the reproductive mechanism of viruses. The suggestion has been made that surface tension would make the direct fission of such minute objects impossible, and that relatively large reproductive bodies are formed, from which new virus-particles bud off. Bodies of this description have been demonstrated in virus-fluids by ultra-violet photography, but their significance remains in doubt.

It is highly improbable that viruses are generated spontaneously, but it may perhaps be worth a passing mention that Pasteur's complete destruction of the theory of spontaneous generation took no account of ultramicroscopic life; so that the question, so far as the viruses are concerned, may be considered as still open.

§ 3. INFECTIVITY

Extremely small quantities of virus are able to cause infection. Thus a 1 in 10,000 dilution in water of the juice of a tobacco-plant infected with mosaic disease will transmit the infection when applied to a wound of a healthy plant. The virus of polyhedral disease of caterpillars will sometimes infect at a dilution of one in a million. Again, the British and American commissions on foot-and-mouth disease found infective material still active at a dilution of one in ten million.

Some plant viruses are so infectious that minute wounds such as needle-pricks are all that is necessary to start the disease; but an entirely unwounded plant cannot be infected. To other viruses, however, the tissues of the host-species seem to have a high average resistance, transmission experiments being only successful in a small fraction of the attempts; and in certain diseases of this group, experimental transmission, except by grafting, has always failed, although there is little doubt that the virus is normally carried and introduced into the healthy plant by insects.

Multiplication or some other development of the virus undoubtedly occurs in the carrying insect. For instance, after *Eutettix tenulus* has fed on a beet-plant suffering from curly-top, it is unable to infect a fresh plant until one or two days have passed. In the case of the leaf-hopper, *Cicadula sexnotata*, which carries the virus of aster yellows, careful experiment has proved the incubation period to be at least ten days. The duration of infectivity varies in different cases from a few days to several months. The health of the carriers appears to be unaffected.

There is a considerable, but far from absolute specificity in the insect-virus-plant complex. Some viruses, for instance, may be transmitted by several species of insects, which are, however, all of the group of the Homoptera. One insect, again, may disseminate several viruses. It is clear that the geographical distribution of any virus disease must depend primarily on the prevalence of its insect-carriers, just as the distribution of typhus fever depends on that of the human blood-sucking louse.

Dissemination of Viruses. Generally speaking, the modes of dissemination are the same as for bacteria: droplets of saliva, traces of excreta, infected particles

of skin moving about as dust. Transportation by insects also plays a large part. It has been suggested with some reason that viruses may be spread with especial ease by the wind.

Concerning the *mechanism of infection* little or nothing is known, though an interesting beginning has been made in America by the discovery that the filterable agent of a transmissible connective-tissue tumour (sarcoma) of chickens has a combining affinity for the muscle-pulp of chickens, but none for the corresponding tissue of refractory animals such as pigeon and rabbit. The brain and the liver of chickens are also devoid of combining power. Further, the embryo, if injected with the virus, shows selective tumour-formation in the mesodermal layer. The tumour-viruses, however, are in some respects unique, as will shortly appear.

Some Characteristics of Virus Infections of Plants. Kunkel says the diseases are not local, but general; that is, the whole plant is infected. The pathological process is, however, closely associated with growth and development (cf. bacteriophage, p. 81), for symptoms of disease are manifested only at or near growing-points, and seldom in tissues that are mature when infection takes place. Local symptoms do not appear at the point where a plant is inoculated, unless this is done at a growing-point. There are a few exceptions to this rule, which none the less holds good in the majority of cases.

It may be mentioned here that other viruses also seem to have a special liking for the immature organism or growing tissue. Sacbrood of bees and the polyhedral disease of lepidoptera attacks only the larval forms. A large group of animal viruses (e.g. variola, herpes, foot-and-mouth disease and many others) have a predilection

for the actively growing layers of skin and mucous membranes, particularly in cases where rapid growth has been caused by damage. Further, the vaccinia virus and virus III of rabbits, will grow in a transplantable rabbit-tumour and survive longer than in the tissues of the healthy animal. Since the visible action of the bacteriophage is practically confined to bacterial cells in the phase of rapid multiplication, some authors have used it as a further illustration of this principle.

§ 4. TRANSMISSIBLE TUMOURS OF FOWLS

In 1911 Peyton Rous investigated a tumour that appeared spontaneously in one of his chickens kept for experimental purposes. This growth was of the connective-tissues series resembling the spindle-celled sarcoma of man.

Rous removed the tumour, minced and ground up a piece in some fluid, filtered it through porcelain and injected the cell-free filtrate into other fowls of the same brood. After a few weeks each of them developed a tumour of precisely the same histological character as the original. At first only blood-relations of the parent fowl were susceptible to infection, but after some passages the active agent became less particular, and at the present time any variety of domestic fowl will 'take'.

This remarkable discovery stimulated others to watch for spontaneous tumours of fowls, and it was not long before a good number were observed and proved to be similarly transmissible by filtrates. The remarkable thing is that their various histological characters may be perfectly distinct and are always faithfully reproduced in the newly infected fowl. Not only the characters of the chief cells, but the structure of the tumour, its rapidity of growth and tendency to regression, are

all constant and characteristic. One tumour is, as we have said, a spindle-sarcoma, another an osteo-chondro-sarcoma (bone, cartilage and fibroblastic cells), a third an endothelioma. The differences, therefore, are profound.

The number of these tumours—not, of course, all histologically different—now runs into many dozens. Specificity for the fowl is a constant quality. The potency of filtrates is very variable, both with different tumours, and in different experiments with a single tumour. Some types come near in this respect to the non-filterable tumours of mammals, which can only be transmitted by transplantation of cells.

We have here, then, a unique phenomenon: the appearance and progressive multiplication of a special type of cell caused by the introduction of a few drops of watery fluid which is derived from cells of the same type. Though it is apparently of the same order of phenomena as virus-infections, its histological aspect is so completely without parallel that no satisfactory explanation has been forthcoming. If, however, we take the facts at their face-value, we can hardly avoid the conclusion that something ultramicroscopic can be liberated from a disintegrated cell and can impart the properties of the latter to other cells with which it comes into contact. Hitherto we have only known inheritance by continuity, but here we seem to have inheritance by discontinuity. Are the ultramicroscopic transmissible factors of the nature of genes? We shall see later that the same question arises in connexion with the bacteriophage, and we will postpone further discussion till that subject has been fully dealt with. Incidentally, the transmission of determinant factors from a defective mutant cell to normal cells would, if established, throw a flood of light on the problem of human cancer; and it seems that many cancer-

researchers are at the present moment turning their eyes hopefully in this direction.

§ 5. INTRACELLULAR BODIES IN VIRUS DISEASES

One of the earliest results of the study of the group of diseases that we now attribute to the viruses was the discovery of abnormal objects in the infected cells. At first these objects were named after their discoverer or first complete describer, thus: 'Guarnieri bodies' in small-pox and vaccinia; 'Negri bodies' in rabies, and so on. As the number of diseases showing these structures increased, the terms of 'cell-inclusions' or 'inclusion-bodies' came into use. These intracellular bodies are of irregular size, shape and number; they are found only in virus-infected tissues, including tissue-cultures, and may occupy either the cytoplasm or the nucleus, or both (Fig. 14). They are usually found early in the illness, and may disappear in the later stages. Usually quite small, they may reach a relatively large size, equal to that of the cell-nucleus. They can be stained by Giemsa's method, with Pappenheim's stain, with Heidenhain's iron-haematoxylin or with simple haematoxylin and eosin. Their coloration is generally distinct from that of chromatin. They occur as frequently in plant virus-diseases as in those of animals, with the important exception of the chicken-tumours; and they are essentially alike in both. They can be produced experimentally by inoculating a susceptible animal or plant with a filtrate containing living virus without cells or other visible objects. In animals they are most often seen in the growing layers of skin and mucous membranes, but they may be found in any infected tissue. It was at first thought that they were protozoa, reproducing by means of a filtrable form.

This notion has now been abandoned, and it seems most likely that they are masses of virus encased in a lipo-protein material either secreted by the virus or made by the cell-protoplasm. Giemsa's stain sometimes differentiates spherical granules from the surrounding sub-

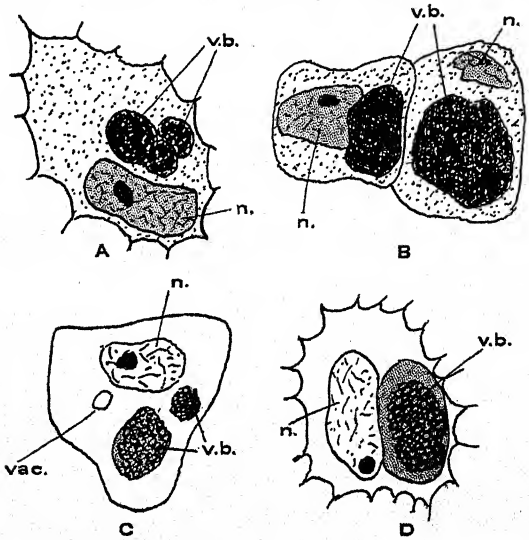

FIG. 14.—Intracellular virus-bodies. Epidermal and corneal cells of chicken and rat infected with vaccinia virus. After Ludford (Semidiagrammatic)

n = nuclus ; *vb* = virus-body ; *vac* = vacuole.

stance, and it has been shown that if the cell inclusions in fowl-pox are crushed, they appear to consist of minute bodies of regular size embedded in a hyaline substance. V. Prowazak coined the names ' Chlamydozoa ' (cloaked animals) for the bodies, and considered it established that they are the virus. As we have already seen,

recent researches uphold this view. Inclusion bodies have not been described in all virus infections, but they exist in the majority of those best studied, and it seems probable that changes of the kind are characteristic of that class of viruses which is truly (ultra) microbic. They do not occur in bacterial infections or other disorders of tissue. Recent observations on guinea-pigs infected with the salivary-gland virus strongly suggest that it is only in functionally active cells that inclusion-bodies are formed. Tying the duct of the gland inhibits their appearance, although the cells are thereby injured, and their resistance presumably weakened.

§ 6. VARIATION AND ADAPTATION

Certain changes of character are known to occur in viruses as the result of altered environment. For example, the human small-pox virus if injected into a calf causes a pustular eruptive disease exactly similar to natural cow-pox. After a sufficient number of successive passages of this kind it loses the power of causing the generalized disease, small-pox, in human beings, and now gives rise only to the local pustules. These are, however, sufficient to protect from subsequent small-pox infection. It is, in fact, just such a cow-adapted virus that is used for human vaccination. Reversion of the virus to full human pathogenicity has been stated to occur on passage through the human body, but only if the virus is recovered in the earliest stages of calf-passage. After one or two complete passages, the modification becomes fixed. Spontaneous derivation of human small-pox from cow-pox has never been recorded.

A second example of this kind of adaptation is given by the virus of fowl-pox. This disease, which occurs in pigeons as well as hens, is readily transmitted from the former to the latter; but strangely enough the hen-

virus will never, or at least hardly ever ' take ' in pigeons. Moreover the pigeon-virus on passage through the hen loses most and sometimes all of its infecting power for the original host.

The pox diseases, indeed, are of the greatest interest from this point of view. Human small-pox is transmissible to cow, horse, goat, sheep, pig and probably hens, and the natural poxes of these animals are transmissible to many of the other animals on the list, though not to man. But the interesting thing is that the spontaneous disease in each species is epidemically infectious, is generalized through the body and is usually severe, whereas the same infection in a related species takes the form of a much more localized mild infection which is seldom if ever transmitted to fellow-beasts of the same or other species.

Some believe that man is the sole source of pox, the virus of which can at any time infect domestic animals. The Middle Ages have left records of simultaneous outbreaks of pox in man and beast, decimating the farmyard along with the farm-house. Others think that there are several epidemic strains of pox virus, which are each fully infective for their true host, but which can adapt themselves to other animals to a limited degree, causing only a mild, local, uninfectious lesion. We have seen that the clearest case of this is the cow-pox derived from human small-pox, and it is of considerable interest that a definite loss or masking of a character is involved; for the virus ceases to be able to cause the original type of illness in its first host. It appears that ' epidemic ' sheep-pox behaves in the same way when inoculated into a goat or cow, and that there may here be a general law for the behaviour of viruses of this genus, involving a process parallel to mutation by loss of a gene.

The viruses, then, seem to have the power of rapid adaptation. Rapidity, however, is a relative term, whose significance depends on whether the generation-time of the organism is measured in minutes, months or years. The meaning of 'permanence' is clearly affected by the same considerations. Since a bacterium or virus can produce as many 'generations' in twenty-four hours as man can in a thousand years, we can hardly apply the same time-standard of permanence to both. For microscopic life we accept as fixed a modification that persists for some weeks or months in circumstances permitting rapid multiplication and favourable for reversion.

The comparison we have just made between virus and man is, of course, in many ways absurd. If we are to draw any parallel at all between a microscopic asexual creature and a higher animal or plant, it must be between the microbe and the germ-cells, or their nuclei. In the case of viruses, indeed, we must go even lower, perhaps down to the ultimate invisible chromatin grains of which the chromosomes are built. Genes and viruses must be of the same order of magnitude.

Adaptation of similarly rapid course and at least relatively permanent has been observed in certain of the virus diseases of plants. For instance, there are two forms of spontaneous curly-top disease of sugar-beets, one severe and the other mild. If the virus from the severe form be passed through one or other of the species *Chenopodium murale*, *Rumex crispus* or *Suaeda moquini*, it loses much of its virulence, and is able only to cause the mild form of infection in the sugar-beet. In all these instances we seem to have a permanent modification directly caused by environment, occurring with great rapidity and reproducible by experiment. And in this connexion it may be worth

contrasting the sheltered life led by the chromosomes of the germ-cells of higher creatures with the direct exposure of bacterium or virus to external influences.

In the study of virus infections the question of the generic connexion of two similar but distinguishable diseases frequently arises. For example, the mild small-pox or alastrim, now prevalent in England, 'breeds true' in successive epidemics. Its case-mortality varies from 0·3 to 3·0 per cent, whereas that of true small-pox is from 20 to 70 per cent. Is the virus a temporary modification of the small-pox virus, or a fixed and separate variety? Owing to the impossibility of experimenting on human beings, the question lacks a certain answer; but circumstantial evidence points to the derivation of the alastrim-virus from small-pox by mutation. For pure epidemics of mild cases were unknown until quite recent years, and it is impossible to doubt that the disease is an entity—and a new one.

A very similar problem arose when the resemblance of the vesicular stomatitis of cattle to foot-and-mouth disease was observed and studied. The two viruses, which gave rise to symptom-complexes that are usually somewhat different, but may on occasions be almost identical, have the majority of their characters in common, but one or two constant differences. The foot-and-mouth virus refuses to 'take' in horses; the other infects them readily. Inoculation of cattle, guinea-pigs or swine with either virus fails to protect against the other. In such cases as these the two viruses have to be placed provisionally as separate varieties, with the reservation that experience may prove them to be unstable modifications of a single entity.

This particular problem is complicated by the fact that the foot-and-mouth virus has been found to com-

prise two, if not three types, separable by immunity experiments. Thus, viruses A, B and C, obtained from different parts of Germany, can be injected successively into the same animal and each give rise to a typical attack; whereas a single attack with one of the types protects completely from subsequent inoculation of that type. Quite recently it has been shown that the three variants become antigenically identical after passage through guinea-pigs; so that they must be merely temporary modifications.

A similar complexity is emerging at the present time from the study of human typhus fever, as it is called in Europe and the indistinguishable 'Rocky Mountain spotted fever' and Mexican 'tabardillo'. The symptoms are identical in the three, but the viruses are distinct, though extremely alike, and may be considered as varieties of one species. Whether such variants arise by discontinuous mutation or by progressive adaptation to slightly different environments (e.g. different breeds of cattle) is a matter of pure speculation; but it is a useful working hypothesis that a virus can fairly readily become adapted, by natural selection, and form relatively stable variants.

Certain recent experiments on plant-mosaic disease have shown how readily modifiable the virus may be.

TABLE II

	Plants in succession	*Mode of inoculation*	*Type of disease*
1.	Potato	—	Mosaic, ordinary.
	Tobacco	Needle	Ringspot.
	Potato	Needle	Severe and very infectious mosaic.
2.	Potato	—	Mosaic, ordinary.
	Tobacco	Aphis	Green line disease.

In Table II are shown the curiously different effects of

transferring potato mosaic disease to the tobacco-plant by two methods of inoculation: the sterile needle, and the living insect. The symptoms of the four diseases resulting are certainly more divergent than those of human small-pox and alastrim. We know of no analogy in bacterial diseases, nor is it easy to imagine how the characters of a virus could be so changed by short sojourn in an Aphis. This section of the experiments is open to the objection that an insect can hardly be considered a clean instrument, and it is difficult to exclude the possibility of a second and different virus being introduced by it.

In another set of experiments the ringspot virus was passed by needle inoculation through successive generations of tobacco-plants, and was found to increase steadily in virulence. Whereas at first it produced only ringed spots, it soon began to cause serious lesions of the leaves, and ended up by producing a severe and fatal general infection of the plant. Further, the virus thus modified, when transferred back to potato gave rise to a violent mosaic infection, which, unlike the natural disease, generally proved fatal.

We have finally to mention the increase of virulence acquired by the human poliomyelitis virus on passage through a susceptible host to which it has not hitherto been adapted. This virus, when fresh from the human subject, kills only 20 to 40 per cent of monkeys, but after a few monkey-passages the mortality rises to nearly 100 per cent. In this, its behaviour is like that of bacteria, and can be theoretically explained by natural selection.

§ 7. ANTIGENIC PROPERTIES AND IMMUNITY

Natural Immunity and Carriers. A species of animal or plant may be completely insusceptible to a virus

disease that attacks an allied species. Further, a most significant fact has been demonstrated by Baur in the genus *Abutilon*. If an infected plant of a susceptible species be grafted with a scion of an immune species, the latter remains healthy. But if the apparently healthy immune scions are then grafted with healthy but susceptible scions, the latter show symptoms of infection. Naturally immune species are thus proved to be able to harbour and transmit a virus without themselves suffering from the disease. A parallel may be drawn with the variable individual immunity to infantile paralysis which is believed to exist in human beings. Transmission by apparently unaffected individuals harbouring the virus (carriers) probably constitutes one of the chief difficulties in preventing the spread of this infection. The presence of bacteriophage in resistant and apparently normal cultures of bacteria (see p. 76) is alleged by some to be a comparable phenomenon, though we have reason to doubt the accuracy of the parallel.

So far as is at present known, most of the animal viruses when injected into animals give rise to antibodies similar to those evoked by bacteria. Specific precipitating and complement-fixing antibodies have been demonstrated in the serum of rabbits injected with the vaccinia-virus. By means of such serum, varicella (chicken-pox) virus can be differentiated from vaccinia (cow-pox) and variola (small-pox), the latter two being indistinguishable.

The serum from persons or animals convalescent from virus-infections, such as herpes, rabies, foot-and-mouth disease, vaccinia or poliomyelitis, has been shown to destroy or weaken the infectivity of the specific virus when the two are mixed and injected into sensitive animals. The union of virus and antibody is at first

unstable, and separable by mere dilution, but after some twenty-four hours it becomes firm.

Prolonged or even lifelong immunity after an attack is, on the whole, characteristic of virus-infections. Although there is no sharp distinction to draw in this respect between viruses and bacteria, one cannot help contrasting the relatively transient protection afforded by most bacterial infections. Modern work tends to find a similar immunity-mechanism in the two cases, but it seems possible that the quantitative difference lies in the virus being a cell-parasite, and giving rise to a more solid tissue-immunity than the bacteria, whose habitat is extracellular. In the latter case circulating antibodies (humoral immunity) probably play a larger part, though they have been demonstrated also in virus diseases. More theoretical, but not without some experimental basis, is the view that the virus continues indefinitely in the body after the illness is past, and renders the whole organism refractory to further attacks. In certain bacterial diseases (tuberculosis of man; *B. aertrycke* infection of mice) prolonged persistence of the bacteria in some organs after recovery from the initial disorder has been shown to be consistent with apparently normal health; and some very recent American work has shown that viruses can be obtained by cataphoresis from tissues that have apparently fully recovered from the effects of an infection undergone four months or more previously.

From the point of view of the parasite, it seems that a completely successful invasion should end in symbiosis; and it is not impossible that plants, animals and man may harbour a variety of viruses adapted to the common life.

The classical example of preventive inoculation against virus diseases is provided by Pasteur's well-

known treatment for rabies (hydrophobia). As early as possible in the very long incubation period after a bite from a mad dog the human subject is treated with a series of injections of living but attenuated virus, which gives him an increasing immunity as the ultramicrobe develops within his tissue; with the result that the disease is either completely aborted, or at least so modified as to cause but little danger. The virus is progressively attenuated for this purpose by drying the spinal cords of artificially infected animals for different lengths of time. A very weak, long-dried virus is first administered, then stronger and stronger ones.

Immunization with 'killed' viruses has, at present, only had very limited success; but recent work raises hopes that temporary protection, such as is obtained with most bacteria, will become feasible before long. For the distemper of dogs a system of active immunization has lately been evolved, which entails a preliminary course of injections with a 'vaccine', consisting of a suspension of tissue of infected guinea-pigs treated with an antiseptic, and then a further injection of fully active virus. The dogs rarely become infected, because of the resistance they have acquired from the preliminary treatment.

Still more recent is the apparently successful use of a similar killed vaccine for the protection of human beings against yellow fever. Here a final treatment with 'live' virus is impossible, owing to the risk it involves.

In two human virus diseases, measles and poliomyelitis (infantile paralysis), a valuable form of protection and curative treatment has been found in the transference of blood-serum from a healthy subject who has recently recovered from the illness to another whom it is desired either to shield from infection or to save from

serious damage when infection has already occurred. It has been best worked out in measles, where success may confidently be expected if the treatment is correctly carried out.

The Sensitization of the Human Body by Viruses to Bacterial Infection. Here and there in medical experience peculiar associations or successions of virus and bacterium arouse speculation whether the ultramicrobes may perhaps prepare the way for microbic invasion. In swine-plague a virus is the originator of the fever, a bacterium (*Salmonella*) the almost constant secondary invader and cause of much of the damage. In typhus fever, a microbe of the genus *Proteus* seems constantly to follow the virus. Again, the influenza problem, and perhaps that of common colds, seem likely to be solved by the association of a virus, the invader, with bacteria that by themselves would be unable to penetrate the tissues. For example, some recent American work has shown that common colds can be transmitted to chimpanzees by means of sterile filtrates of nasal discharge. As soon as the catarrh develops, large numbers of pneumococci (a pathogenic species of *Streptococcus*) are found in the nasal muco-pus. There were presumably a very few of these present in the healthy nose, as there often are in human beings.

In experimental work with vaccinia virus, widespread infection of the animal often occurs with bacteria of the Pasteurella group. Since these are certainly not introduced with the virus, either they must be already lying latent in the animal body, or, if they enter casually after the virus-injection, they must have a far greater virulence for virus-infected than for healthy tissues.

§ 8. LIST OF THE CHIEF DISEASES KNOWN OR BELIEVED TO BE CAUSED BY VIRUSES (INCLUDING RICKETTSIA)

Diseases of Man.

Typhus fever, Rocky Mountain spotted fever, Japanese flood fever, Trench fever, Small-pox (variola), Vaccinia (cow-pox), Alastrim (mild small-pox), Herpes, Chicken-pox, Encephalitis, Poliomyelitis, Psittacosis, Molluscum contagiosum, Warts, Measles (rubeola), German measles (rubella), Influenza (grippe), Common colds, Trachoma, Rabies (hydrophobia), Yellow fever, Dengue.

Horse.

Horse-pox, Pernicious anaemia, Vesicular stomatitis, African horse-sickness.

Cattle.

Cow-pox, Rinderpest (cattle-plague), Foot-and-mouth disease, Papular stomatitis, Heartwater.

Sheep.

Sheep-pox, Blue-tongue (catarrhal fever), Nairobi disease.

Swine.

Swine-pox, Hog-cholera, Foot-and-mouth disease (same as in cattle).

Goat.

Goat-pox.

Dog.

Distemper.

Rabbit.

Virus III infection, Infectious myxomatosis.

Guinea-pig.

Salivary disease, Paralysis.

Mouse.

Sarcoma, Infectious ectromelia.

Birds.

Fowl-pox (contagious epithelioma and diphtheritic roup), Fowl and Blackbird plague, Sarcoma of chickens, Leukaemia of chickens.

Fish.

Carp-pox, Infectious epithelioma, Lymphocystic disease.

Insects.

Bees : Sacbrood.

Moths : Wilt or polyhedral diseases of 12 species of 7 families ; including Jaundice (Grasserie) of silk-worms.

Butterflies : Wilt, of 2 species ; Two unnamed diseases of the cabbage butterfly.

Plants.

Mosaic diseases of more than 160 species of at least 32 families.

also

Potato Streak : Sprain, Leafroll, Spindle-tuber, Witches broom.

Strawberry : Witches broom, Yellows.

Raspberry : Streak, Curl.

Cranberry : False-blossom.

Wheat : Rosette.

Peach : Yellows, Rosette, Little peach.

Sugar-cane : Sereh disease, Fiji disease.

Banana : Bunchy-top.

Pea-nut : Rosette.

Beet : Curly-top.

Tomato : Spotted wilt.

Hops : Nettle-head.

Sandal : Spike.
Asters : (china) Yellows.
Cotton : Crazy-top.
Spinach : Blight.

Note.—In many of these plant-diseases the causal agent is assumed to be a virus because of the similarity of the disease to diseases of proved virus-origin, and because thorough modern investigation has failed to trace a bacterial cause.

To those who wish to pursue the study of the ultramicrobes the following treatises are recommended :

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LIPPSCHÜTZ, B., 1929, in *Handbuch der Pathogenen Mikrooganismen*. Kolle, Kraus und Uhlenhuth, viii, 311.

RIVERS, T. M., 1928, *Filterable Viruses*. London.

System of Bacteriology, Medical Research Council, 1930, vol. 7, London.

TOPLEY, W. W. C. & Wilson, G. S., 1929, *The Principles of Bacteriology and Immunity*. London, E. Arnold.

PART III

THE TWORT-D'HERELLE PHENOMENON OR 'THE BACTERIOPHAGE'

§ 1. DISCOVERY: DESCRIPTION: THEORIES

NO bacteriological discovery in the last twenty-five years has aroused such a widespread interest as that of the mysterious agent that destroys bacteria and regenerates itself in the process. Though the phenomenon had undoubtedly been seen by Gamaleia in 1899, the first proper description of its fundamental characters was given by F. W. Twort in 1915. Two years later it was independently described by d'Herelle, who was more fortunate than Twort in having the opportunity to investigate it thoroughly. Twort had been attempting for some time to discover non-pathogenic filterable viruses, in the external world, but failing to do so, turned to materials derived from animals. In a culture on agar-gelly of a white Staphylococcus, grown from the calf-lymph used for vaccination, he saw small patches where the film of microbes seemed to have been clarified or dissolved. Having filterable viruses in mind, he took an ordinary culture of the coccus in a fluid medium, filtered it through porcelain to remove all the cells, and mixed a small quantity of the filtrate with another suspension of the same organism. A culture made from this, after starting to grow, became completely clarified, and very few of the microbes in it remained alive. Thus it was proved that the active agent

passed through the filter. Tested on other cultures of micrococci from different sources, the filtrate showed an ability to dissolve them to a certain degree.

Being prevented by the turmoil of war from pursuing the matter further, Twort confined his speculations to an open statement of the alternative explanations, inclining perhaps a little to the view that the principle originated in, rather than outside, the microbes. Since the study of bare spots on culture-tubes did not seem likely to be of any assistance in winning the war, the scientific world paid but scanty attention to this important piece of work.

The following year, however, an independent discovery of what is clearly the same phenomenon was made by d'Herelle, who had been investigating an infectious bacterial disease of locusts in Mexico, and had noticed now and then a strange irregularity or 'inhibition' of the growth of the bacteria on solid culture-media. He was at that time influenced by the theory that this and many other infections of animals and man might be caused primarily by an invisible virus, the alleged specific bacteria being really only secondary invaders. If this were true, cultures of the microbes isolated from the infected subject might contain the virus, which was perhaps responsible for inhibition of the bacterial growth. As soon as he had an opportunity, he put the theory to the test, by examining a series of cultures from cases of human bacillary dysentery. We will give his own account of what he found:

'In August 1916 an adult with a severe bacillary dysentery (Shiga's bacillus) was under treatment at the Pasteur Hospital. Each day about 10 drops of the stool (i.e. faeces) were collected and placed in a tube of bouillon. After incubation overnight the suspension was filtered through a Chamberland candle. Into some bouillon, previously inoculated with Shiga bacilli, about 10

drops of this filtrate were placed, and the material was returned to the incubator at 37° C.

'Throughout the duration of the disease, all of the tubes, prepared each day in the same manner, gave normal cultures of *B. dysenteriae*. One day, the tube prepared the day before remained sterile. Investigation showed that the patient gave evidence of notable improvement, and, as appeared later, this was shortly followed by definite convalescence. To the bouillon thus inoculated, and containing filtrate, and which had remained to all appearances sterile, a suspension of Shiga bacilli derived from a fresh agar culture was added to yield a marked turbidity. This tube was placed in the incubator. After about 10 hours it was again clear. . . . A drop of the dissolved culture was added to a fresh bouillon culture of Shiga bacilli. About 15 hours later the bouillon was clear, all of the bacilli originally present had been dissolved. Thus, several successive passages were effected in the same way, employing each time a drop of the culture previously dissolved added to a fresh culture of Shiga bacilli. In this repetition of the process, instead of becoming weaker, the activity became more and more pronounced; that is, the disappearance of the bacilli was effected with greater and greater rapidity.'

In this manner, starting with a drop or two of a lytic filtrate, more than a thousand successive 'passages' in bacterial cultures were carried out, each passage diluting the original quantity of filtrate some hundred-fold, and at the end the final filtrate was so active that a billionth part of a cubic centimetre completely lysed a culture of 2,000 million bacilli.

Quite as interesting was the behaviour of the filtrate-treated cultures on solid media. To a well-grown young Shiga culture in broth a tiny amount (about a ten-thousandth of a c.cm.) of filtrate was added, and a small drop of the mixture was immediately spread on the sloped surface of a tube of agar. The mixture was then put in the incubator, and drops were similarly spread at intervals of an hour. Incubation of these sub-cultures revealed a very interesting series of irregularities. The first, which had been spread immediately, showed an

almost continuous normal film of growth, except for two little bare circular islands or 'plaques' (see Fig. 15). The second, made after an hour's incubation of the mixture, had six plaques; the third, made after two hours, about a hundred. The subculture made after four hours' incubation was completely bare.

What could be the explanation of this strange spon-

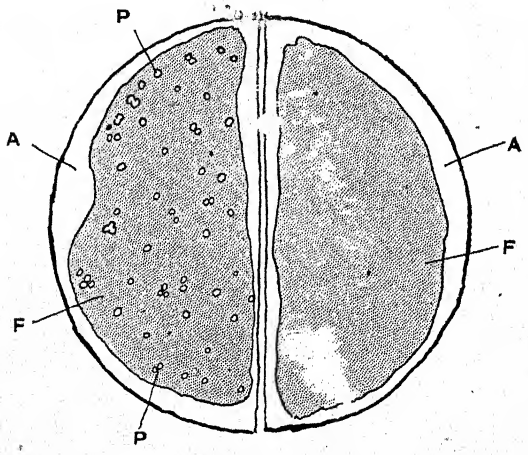


FIG. 15.—Bacteriophage-action on a Surface

A = Agar-medium. F = Film of bacterial growth. P = Plaques of lysis (Diagrammatic).

Left side = Action of bacteriophage. Right side: control growth, without bacteriophage.

taneous transmissible lysis of living bacteria? Ordinary bacterial lysis was well known to occur spontaneously to a limited degree in old cultures, or to be readily induced by certain chemical and physical agents, and by specific antibodies in the blood of immune animals; but it differed fundamentally from this new form of lysis in being entirely non-transmissible. The discrete arrange-

ment of the plaques on agar cultures treated with a high dilution of the transmissible lysin led d'Herelle to believe that he was dealing with a corpuscular agent rather than a substance in solution. The fact that an infinitesimal trace of filtrate could, in unending series, confer lytic power on unlimited quantities of culture made him visualize the agent as a living, self-propagating virus or ultramicrobe. It seemed, in fact, to be a destructive parasite of bacteria—aptly to be named bacteriophage.

D'Herelle's thorough work and his uncompromising advocacy of the virus-theory stimulated many bacteriologists to probe further into the matter, and before long a strong rival school of thought developed under the leadership of Jules Bordet. To these workers the transmissible agent does not appear as an independent living entity, but as a peculiar product of the bacterial cell. To some it is an enzyme, or proenzyme, catalysing the autolysis of the cell; to others, a growth-promoting hormone, dislocated from its natural place and acting catastrophically. In both cases the agent is pictured as present in the normal cell and taking part in the natural process of growth, but also as capable of becoming abnormally active under the influence of certain disturbing stimuli and of thus causing the destruction of the cell. Liberated in this way into the surrounding fluid, it induces a similar metabolic vitiation in other cells with which it comes into contact.

A variant of this theory advanced in slightly different forms by Wollman, Bail and others is that the transmissible factor is not directly concerned with the autolysis, but is a hereditary factor or 'gene', determining self-destruction, which is liberated extracellularly by mutant, defective, autolysing cells, and which by penetrating normal individuals confers on them the suicidal constitution.

These three main theories have been shortly stated here in order that the reader may judge between them as he peruses the following pages.

§ 2. THE GENESIS OF THE TRANSMISSIBLE LYSIN

Origin from Animals and their Products. The intestines of animals and man contain something that induces transmissible lysis in suitable strains of bacteria, commonly belonging to the coli-typhoid-dysentery group of the genus *Bacterium*. According to d'Herelle, this is true of every animal and every man. However this may be, it is agreed that domestic animals, and especially the pig, are reliable sources. To obtain a transmissible lysin a small portion of faeces is put in a flask of nutrient broth which has already been sown with the bacillus (e.g. *B. coli* or *B. dysenteriae*). After incubation for a night the culture is filtered through paper and kieselguhr, and the filtrate is passed through a porcelain or a Seitz asbestos filter. The resulting fluid will betray the presence of bacteriophage, if a susceptible bacillus has been used. It may be necessary to try a considerable number of species or races before finding the right one, which is often the preponderating type of *B. coli* in the animal's own intestine.

The blood of animals and man, particularly during recovery from infectious disease, sometimes produces bacteriophage, and cultures of bacteria injected into the peritoneal cavity of healthy guinea-pigs may acquire lysinogenetic properties.

It seems that the foetus and new-born child are devoid of this power, which first appears after about a week from birth, afterwards to persist for life.

Inanimate Materials. Sewage, many specimens of soil, and water from rivers, streams or even taps are

lysinogenetic. In 1896 Hankin observed that the water of the River Jumna immediately below Agra contained upwards of 100,000 bacteria per cubic centimetre ; a few miles lower down less than a thousandth of that number could be found. This is now believed to be the earliest recorded instance of bacteriophage action. Since all the sources mentioned under this heading are subject to contamination with excreta, they should perhaps be included in our first category of sources.

The property has also been occasionally demonstrated in commercial preparations of enzymes, such as pancreatin and trypsin ; but it is certainly not the enzyme itself that acts in such cases.

According to Hadley, heating to 120° C. (moist) for twenty minutes does not abolish their activity. This very important observation stands in need of confirmation. Finally, it has been reported that sterile distilled water may initiate bacteriophagy, but it is uncertain whether anything more than a spontaneous lysinogenesis of the bacteria can be inferred from such experiments.

Bacterial Cultures. Under certain conditions the lytic principle can be demonstrated in stock cultures of bacteria that have been growing in the laboratory for years without any known contact with bacteriophage. Some cultures indeed are from the first openly and constantly lysinogenetic, growing always in the form of moth-eaten colonies (Flatterformen. See Fig. 16). Here every colony, and perhaps every cell is involved, and the cultures refuse to yield resistant variants. In most cases, however, in order to demonstrate their lysinogenetic power the cultures have to be subjected to a definite process, consisting of culturing in broth, incubating and filtering (or heating to 60°), then adding a little of the filtrate to a fresh broth culture, and repeating the whole process. After several such passages the final

filtrate is tested on a considerable range of bacteria of the same and allied species, for it is impossible to foretell the precise affinities of the phage.

The proportion of old stock cultures that can be proved to be lysinogenetic in this way varies considerably. In one batch as many as twelve out of twenty-one of the typhoid-coli-dysentery group were positive, but most workers have only had from two to five per cent of

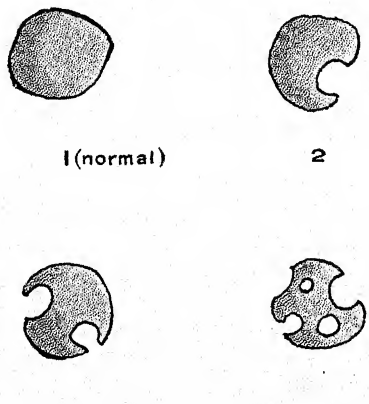


FIG. 16.—Nibbled or Moth-eaten Colonies (2, 3, 4)

successes. Irregularities of lysinogenesis are sometimes encountered; for instance, cultures have been recorded as producing bacteriophage when grown on agar containing lithium-chloride, but none when cultivated on plain agar.

Further, Bordet has demonstrated the disappearance and reacquisition of lysinogenetic power in a culture of *Bact. coli*. This culture, heated to 57·5° C. and filtered, induced lysis both in itself and in *Bact. dysenteriae*

(Shiga). But when a number of colonies were isolated by spreading out on agar, many of them were found completely non-lysinogenetic for the dysentery bacterium. Subcultures from these colonies were carried on for eight months and then re-tested; when, surprisingly enough, most of them proved as lysinogenetic for *Bact. dysenteriae* as the parent culture had been.

(According to the virus-theory, the living bacteriophage is ubiquitous, but is to be found with special ease wherever bacteria have been growing. In the living animal body it diffuses out from the dense bacterial population of the intestinal tract.) If a stock culture in a laboratory proves, on testing, to be lysinogenetic, it is held that the bacteriophage must have been there, living in symbiosis with the microbes, from the first isolation of the culture. Again, if a specimen of pancreatic extract is found capable of initiating the phenomenon, the phage must have been lurking in the pancreas from which the extract was made. This theory accounts for most of the facts quite satisfactorily, but it does not seem capable of explaining how any material can act lysinogenetically after moist heat at 120°—a temperature that rapidly destroys all known living things. Nor is it easy to understand the disappearance and reappearance of the agent in cultures such as those described by Bordet. These facts, on the contrary, are explicable if the lysin originates in the bacteria used for demonstrating its presence. A number of agencies of various kinds may be conceived as stimulating variation of the bacteria, whereby an autolysing variant is produced which is capable of transmitting its weakness to other cells by means of something it liberates into the surrounding medium. Lysinogenetic materials, such as filtrates of sewage, may act either as prime stimulants to variation, or by virtue of a transmissible lysin previously derived

from autolyzing bacterial variants. Hadley and his colleagues have observed that in stock cultures tested for lysinogenesis in the way described above, a pronounced variation of the bacteria *precedes* the appearance of the lysin.

We may add that d'Herelle's hypothesis of the ubiquity of the bacteriophage, which entails its constant presence in the blood and tissues of animals, involves an *a priori* improbability. For since it must be composed of foreign and presumably protein matter, all experience tells us that it should be rapidly eliminated from the organism; and it has in fact been shown by direct experiments that this is what happens when the bacteriophage is injected into animals.

The Species of Bacteria in which Bacteriophagy occurs. In addition to the intestinal bacteria such as *Bact. typhosum*, *Bact. coli*, the dysentery bacterium and the *Salmonella* group, the following organisms have been shown to undergo bacteriophagy: *Staphylococcus*, the *Pasteurella* group, *Vib. cholerae* and other vibrios, *Streptococci*, *Bac. subtilis*, *Corynebacterium diphtheriae*, *Proteus*, *Pseudomonas pyocyanea*, *Rhizobium leguminosarum* and various bacteria that cause diseases in plants. Finally there are representatives of the thermophilic and of the psychrophilic groups of organisms. Doubtless many others will be included as our knowledge of the subject progresses.

The case of *Pseudomonas pyocyanea* deserves special consideration. The common type of this organism produces a blue-green fluorescent pigment, pyocyanin, but non-pigmented variants are common. The normal type of growth regularly shows a modified form of bacteriophagy, or autolysis in plaques, which differs from the classical picture in the group of *Bact. coli* and *Bact. dysenteriae* by its regular hereditary quality. The

addition of filtrates of such cultures to fresh growth does not increase the autolysis of the latter. We seem to be dealing here with a constantly-occurring lethal variation. Occasionally, however, if we can believe the reports, this passes over spontaneously into typical transmissible lysis. D'Herelle refuses to admit this phenomenon into the category of bacteriophagy at all, thereby tacitly confessing that it presents a formidable difficulty to the virus-theory. A somewhat similar non-transmissible lysis is seen in *Bac. anthracis*. We must remember in this connexion that failure of transmission is by no means rare even with the classical phages of the coli and dysentery groups of bacilli.

There seems, in fact, to be a graded series of lytic processes, which should rather be classed together than artificially separated in the attempt to establish a particular theoretical interpretation of a section of them.

§ 3. COURSE AND CONDITIONS OF THE REACTION

When we speak of 'the reaction' we do not mean only the lysis of bacteria, but also the very important phenomena that precede it. Indeed, there is much difference of opinion about the importance of the actual lysis. D'Herelle takes the view that it is the essence of the process, since he believes that 'the virus' multiplies only when solution of the bacteria takes place. But there is some evidence that regeneration of bacteriophage can precede lysis, being bound up rather with the accelerated pre-lytic multiplication of the bacteria than with their subsequent solution.

Moreover d'Herelle himself showed that the phage can persist indefinitely in certain resistent lysinogenetic cultures (see later, p. 99), explaining it as an instance of symbiosis. But this persistence clearly entails regenera-

tion, for otherwise the bacteriophage would necessarily be eliminated by the repeated subculturing of the bacterium. Whether or no lysis is necessary for the regeneration of the bacteriophage, there is no doubt that bacterial multiplication is. On bacteria suspended in water or in any other fluid that does not offer the nourishment necessary for growth, the strongest bacteriophage has no appreciable effect. But let a little meat infusion be added to the mixture, and the reaction proceeds forthwith. Again, if a small, but fully active dose of phage is introduced into two suspensions of a sensitive bacterium in fresh broth, one being quite thin and the other very dense, the former will show the typical lysis and regeneration of the phage, whereas nothing at all can be seen to happen in the latter. The reason is that there is plenty of nourishment for the smaller number of bacteria in the first suspension, so that they grow and multiply freely; but in the second suspension the first stages of growth of the very numerous cells exhaust the medium, and the culture fails to reach the stage of unrestricted multiplication which is necessary for bacteriophagy. This 'Critical phase' of growth is the same as the logarithmic phase described elsewhere (Fig. 5, p. 12). There is undoubtedly an important difference between a cell in this phase and a mature resting cell, though we can hardly claim to know precisely wherein the difference lies. A relative increase of permeability of the membrane, favouring the rapid passage of food inwards and excreta outwards seems the likeliest hypothesis, and such a state would facilitate the entrance of a harmful agent; or the delicacy of the membrane would make it more vulnerable to destructive action from without.

It has been hotly debated whether cells in any other phase of growth can undergo lysis. Without going in detail into the evidence we can state that the restriction

is not absolute. Suspensions containing a fair proportion of mature and even dead individuals may be completely dissolved if there are also numerous actively growing ones in the process of dissolution. The nascent lytic principle can thus attack cells that would be completely refractory under static conditions.

The virus theory offers no adequate explanation of these phenomena. Indeed it is improbable that a virus should have such a very limited mode of action. It is true that many of the pathogenic viruses display a certain preference for young and actively growing tissues (see p. 52), but there is no suggestion that they can only infect cells in active division. On the contrary, if the lytic principle is produced by the cells themselves, it is natural that it should only be generated in the phase of greatest metabolic activity.

If the bacterial population is counted from time to time during the progress of bacteriophagy in a fluid medium, it is often found that waves of multiplication are succeeded by waves of dissolution. As the bacteriophage grows in strength the destructive process outruns the reproductive powers of the bacteria, until finally the merest remnant of the population survives. As we shall shortly see, a complete sterilization of the culture is a rare event, for there are nearly always some individual cells that stoutly resist the attack of the phage.

The course of the reaction varies to a considerable degree in different genera of bacteria. For example, Twort's original description of the lysis of his staphylococcus differs in detail from d'Herelle's observations on the dysentery and colon bacteria; so much so that the latter thinks fit to deny the fundamental identity of the two phenomena. The spontaneous lysis of *Bacillus anthracis*, as we have already seen, closely resembles bacteriophagy, but is nontransmissible; and transmis-

sibility is exceptional in the commonly-occurring spontaneous ' autolysis ' of *Pseud. pyocyanea.*

§ 4. THE PHYSICAL AND CHEMICAL BEHAVIOUR OF THE BACTERIOPHAGE

Under the best conditions the lytic principle passes through porcelain filters, even those of small average pore-size, with little or no loss of strength. By ' ultra-filtration ' through graded collodion membranes it has been estimated that the size of the particles of which the phage consists or on which it is adsorbed, is similar to that of the particles of colloidal silver, or of serum-globulin, i.e. of the order of 20 mμ, or one fifty-thousandth of a millimetre. A given membrane may pass a fraction of the phage and retain the remainder, and Bronfenbrenner showed that if the retained part is washed from the membrane with broth and refiltered, some of it will now pass through. He regards this as showing that the phage is merely adsorbed on colloid particles of varying size. A similar phenomenon occurs in the filtration of certain viruses, the passage of which is much facilitated by the use of broth (a fine, non-viscous colloidal solution). Purified preparations of bacteriophage have been obtained by Bronfenbrenner by ' electrodialysis ', which cannot be proved to contain any nitrogen, and which are not affected by 90 per cent alcohol. This has, moreover, been recently confirmed by some Japanese workers. Thus, although it is held by most experts that the phage is corpuscular and that the particles are of protein, yet it cannot be said that either of these propositions has been proved. The phage undoubtedly behaves in ultrafiltration experiments as a suspension, but this may only mean that the active principle is normally adsorbed on particles of colloid matter.

The electrical charge of the particles appears to be negative when the *p*H of the fluid ranges from 3·4 to 9·0 ; below 3·4 it is positive. The bacteriophage is not volatile, nor does it diffuse at a measurable rate through agar-gel.

According to d'Herelle, centrifugalization of active filtrates effects a concentration in the lower layers, which proves the agent to be corpuscular ; but Bordet was entirely unable to confirm the experiments, the interpretation of which must therefore remain in doubt. The Hydrogen-ion concentrations in which the reaction is at its best range from 7·2 to 8·2, which range is precisely the optimum for the growth of bacteria. The temperature also follows the requirements of the microbes, with an optimum at about 37° C. Indeed it follows necessarily from what has been said in the last section that the best conditions for bacteriophage and for rapid bacterial multiplication are identical. If further proof were needed, it would be found in the fact that thermophilic bacteria, which grow at 60°–70° C., i.e. a temperature that rapidly kills most bacteria, are subject to transmissible lysis at those temperatures only. Similarly the psychrophilic organisms that thrive at a few degrees above the freezing-point of water have their proper bacteriophages, whose action is restricted to these low temperatures, at which the growth and bacteriophagy of ordinary bacteria are completely inhibited. It is hard to conceive of a single species of ultramicrobe that should parasitize and destroy its various hosts at such very different temperatures ; but of course there may be any number of species or varieties, some of which are adapted to special conditions, just as happens with bacteria.

As one would expect from the foregoing facts, there is no constant *thermal death-point* for bacteriophage in general. But it is of great practical importance that the

common bacteria, thriving between 20° and 40° C., are killed by a temperature that does not affect the lytic principle acting on them. Thus if a mixture of dysentery or colon bacteria with bacteriophage be heated to 58° or 60° C. until all the bacteria are dead, the lytic principle will survive, usually with little or no impairment. This procedure is a common alternative to filtration for obtaining pure bacteriophage. Heating to 70° or 75° C. usually destroys the lytic principle. In this connexion it should be remembered that the spores of bacteria often withstand 100° C. (moist) for several hours, whereas the vegetative forms mostly die in half to one hour at 60° C. At temperatures slightly below 70° C. the phage is weakened and produces fewer plaques per unit volume.

It is of some interest that these plaques are of normal size, from which Bordet infers that the heat acts by coagulation on the phage-carrying colloid particles. In its powers of *resistance to antiseptic substances*, such as phenol, perchloride of mercury, alcohol, glycerin, etc., the bacteriophage is intermediate between the vegetative cells and the spores of bacteria ; and in this it resembles the viruses. The effects of chloropicrine, which readily kills bacteria, but does not inactivate ferments, were tested by Wollman on (1) trypsin, (2) bacteriophage, and (3) non-sporing bacteria, with the result that the phage was found much more resistant than the microbes, but less than the trypsin.

The effects of various concentrations of metallic salts have recently been investigated by Kleineberger, with curious results. The most interesting were obtained with sodium and potassium chloride. High concentrations of these salts (molar or higher) have no deleterious action on the bacteriophage, but as the solution becomes weaker, e.g. to $\frac{1}{8}$ or $\frac{1}{16}$ molar, the potency of the

phage diminishes progressively, only to recover again with a further reduction of the salt-concentration. Thus a phage may produce 3,000 plaques when it has been exposed to a molar solution of NaCl, and only ten to twenty with a solution sixteen times weaker. In plain water its potency is 2,000—3,000. This is quite different from the behaviour of bacteria and other microscopic creatures, which are progressively damaged by increasing concentrations of salt; but there are, so far as we know, no data available as to the behaviour of viruses under like conditions.

§ 5. MECHANISM OF THE REACTION

Fixation. If a definite quantity of phage is added to a sensitive bacterial suspension and the mixture is left for about a quarter of an hour and then centrifugalized, the clear supernatant fluid will be found to have lost nearly all its lytic power. In other words, the bacteriophage has attached itself to the bacteria and has been removed by them from the fluid. Susceptible bacteria fix the phage whether they are alive or dead, but lysis follows only in a living culture.

A race of bacteria that is insensitive to a particular bacteriophage has no fixing power for it. Further, if a phage has only a feeble action on a certain bacterium, a large number of cells are needed to fix it, whereas a strong phage needs far fewer. There can be little doubt that this is because the ' weakness ' of a phage in respect to a particular culture is an expression of the small proportion of the cells on which it can act, for when the fixing cells are few, a large amount of culture will be needed to effect complete fixation. On the contrary, ' strength ' means affinity for all the cells; wherefore a relatively small amount of culture will fix a strong phage completely.

In this fixation-affinity recent workers, especially Burnet, see a fruitful analogy with the phenomena of antigen-antibody combination. Specific physico-chemical constitution determines the fixation in both cases. We have even come so far as knowing in certain cases which of the antigenic constituents of the bacteria combine with the phage. In the typhoid-coli group, for instance, there is good evidence that it is the heat-stable antigen (see p. 38).

Fig. 17, modified from Burnet, is a diagrammatic representation of the different fixing powers of the

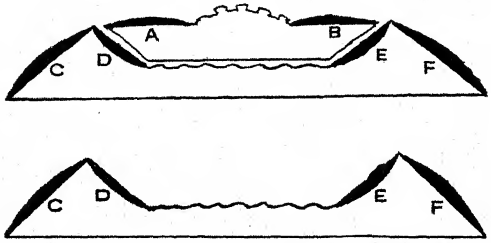


FIG. 17.—After Burnet, 1930

Smooth and Rough phases of a bacterium (see p. 30). Each unit area of the surface of the cell is conceived to consist of a number of different antigens or fixing-areas (thick lines), each with a specific affinity for certain bacteriophages. The Smooth phase is known to have an antigenic carbohydrate complex (specific soluble substance) which is missing in the Rough state. This is represented by the detachable part A B. It has a fixing affinity for bacteriophages A and B only. So long as it is present, the antigens D and E are masked, and the cell shows no affinity for phages D and E, which can only operate on the Rough phase, whose antigens D and E are exposed by the loss of A B. Finally, the fixing

areas C and F are common to, and free to operate in both phases of the bacterium. Hence certain phages act on both Rough and Smooth cultures.

This view of the matter is strongly supported by the discovery that when the heat-stable antigens of two otherwise quite different bacterial species are identical, as they are in *Bact. typhosum* and *Bact. enteritidis*, the range of sensitiveness of these species to different phages is extremely similar. That the range is not necessarily identical does not vitiate the theory, since it is easy to believe that minor variations in the constitution of the antigen may affect its range of phage-sensitiveness without altering its gross serological behaviour. The Smooth-Rough phase-variation is however not the only one that can affect the sensitivity of the bacterium, for there are undoubtedly other antigenic variations which cannot be included in that category, since they do not cause any gross alteration of the character of the culture. For instance, the following table and diagram (Fig. 18), simplified after Burnet (1930), concern a *Staphylococcus albus* from which variants with different phage-sensitivities were derived.

TABLE III

PHAGE REACTIONS OF STAPHYLOCOCCUS VARIANTS

Strain	Phages			Antigenic components
	a	b	c	
Normal	+	+	+	abc
Var. 1	0	+	+	bc
Var. 2	+	0	+	ac
Var. 3	+	+	0	ab

Fig. 18 shows the hypothetical surface-structure of the complete, normal coccus. The variants arise by the

loss of one of the components, a, b, c. The alterations of phage-sensitiveness were actually accompanied by an equivalent antigenic variation, as shown by the different qualities of antiserums made by injecting the variant cultures into rabbits.

This theory of specific fixation cannot claim to be more than tentative, since the phenomena in some groups of bacteria cannot at present be well fitted into the scheme, but it is not likely to be far from the truth since its explanation of the group of facts we have just described is so satisfactory.

The Disintegrative Process. It has been found that the viscosity of a bacterial suspension increases five- or

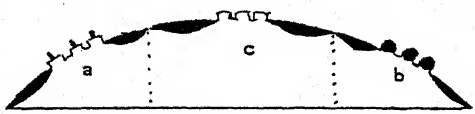


FIG. 18.—After Burnet, 1930

six-fold under the action of bacteriophage. The experiments were done by Bronfenbrenner to discover whether any significant swelling of the cells by imbibition of water occurs as a preliminary to lysis. Since a mass of cells swollen with water occupies a larger part of the volume of the suspension than the same number of relatively dry cells, the viscosity of the fluid must thereby be increased. The rise actually continues until lysis sets in, after which the viscosity drops again to its original value, as cell-disintegration reaches completion. Imbibition of water may thus be presumed to play an important part in the lytic process, as it probably does in other forms of bacteriolysis. It can be explained by an increase of osmotic pressure inside the cell due to a hydrolysis of proteins catalysed by the bacteriophage.

Clear evidence of the breakdown of proteins has, in fact, been obtained by chemical tests in bacteriophaged suspensions of microbes grown in a synthetic medium free from protein.

Direct microscopic observation of the process does not throw much light on its mechanism. It is true that d'Herelle described the entry of granules from without into the cell, their multiplication inside, and their re-discharge at the moment of rupture. These granules he believed to be the ultramicrobe attacking and destroying the cell. Nobody else, however, has been able to confirm this observation; and in any case it is always impossible to prove the nature of granules in an essentially granular fluid. Dead and swollen bacteria often contain numbers of dancing granules, which originate from the cell-protoplasm and have patently nothing to do with the bacteriophage.

The following microscopic phenomena are well established. After a short incubation with a lytic filtrate, the bacteria either swell up, become granular inside, and finally burst, liberating the granular contents and leaving a hardly visible shell, which itself gradually dissolves; or they fade away little by little, without obvious swelling or granulation, and finally disappear, leaving no trace, or perhaps a speck of amorphous debris (Fig. 19). The rate at which these phenomena occur varies with the concentration and the essential potency of the phage. Thus if a strong suspension of dysentery bacilli in nutrient broth, say 250 million per c.cm. is treated with 0·1 c.c. of a bacteriophage of high potency, in thirty to forty minutes a small proportion of the cells begin to show alteration, and in two hours solution is complete with the exception of a very few resistant individuals, which may need a prolonged search to be discovered at all.

If, to a similar suspension, a thousandth part of the

dose of bacteriophage is added (0·0001 c.cm.), the only change observable in two hours is an increase of the density of the suspension, signifying multiplication of the bacteria. Microscopically there are many normal bacilli, and a fair number of unusually long and stout cells; also some swollen, oval or spherical individuals. After three hours partial solution has occurred. Amorphous material is seen in considerable quantity, and the

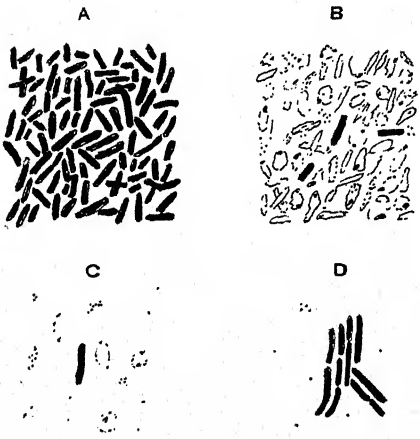


FIG. 19

A, Before. *B*, During. *C*, After lysis. *D*, Secondary growth beginning.

remaining bacilli are large and well formed. Four hours shows a further advance of this process, and in six it is as complete as it was in two hours with the strong dose of phage.

§ 6. POTENCY AND RESISTANCE

Bacteriophages of different origins differ not only in the range of bacterial species, varieties and 'strains' on

which they act, but also in the intensity of their action on a given strain. This variable power is best termed *potency*, though the virus-school, drawing a parallel with pathogenic microbes, favours the term 'Virulence'. The potency range of a phage is characteristic, and stable enough even to allow a classification to be made of a group of phages according to their action on an arbitrarily chosen group of bacterial races; yet it is by no means unchangeable. Potency for a bacterium on which a phage acts feebly can be increased by frequent 'passages', i.e. by repeatedly regenerating the phage on that bacterium.

Opposed to the potency of the phage is the *resistance* of the bacterium which d'Herelle construes—wrongly we believe—as a case of acquired immunity. We have seen that a cell is only lysed if it has an affinity for the phage to which it is exposed. A culture of bacilli may be wholly susceptible, wholly resistant, or mixed; and in the last case its component cells may have all shades of susceptibility and therefore, conversely, of resistance. In a uniformly mixed culture this variable can be theoretically plotted as a probability curve (see Fig. 6, p. 16).

Purification of the bacteriophage. Seeing that filtrates from sewage, animal excreta and so forth may well be suspected of containing a variety of lytic principles, methods of separation have been invented. The first is the method of dilutions. Serial tenfold dilutions of an active filtrate are made, and to each a suspension of a sensitive bacterium in broth is added. On incubation, a point in the series is observed where visible lysis ends. A considerable bulk of suspension is now mixed with filtrate so as to make the concentration of the latter equal to that of the first negative tube of the series. This mixture is then immediately distributed into ten or

more separate tubes, which are put into the incubator. Some of them will undergo lysis; others will show unrestricted growth. It is argued from this that the bacteriophage must be in the form of discrete units or corpuscles, of which there were too few in the mixture to provide one unit for each of the separate tubes. Where lysis occurs it is assumed that a single corpuscle (on an average) was introduced, so that the resulting phage may be considered as pure. Though the supposed proof of corpuscularity is invalid, as we shall see later, the method is justified by its fruits, for the pure phage often differs from its mixed parent, the difference being in the direction of lowered or restricted potency.

A similar purification can be effected by adding a small dose of filtrate to a suspension of bacteria, incubating for a short time, and then spreading a platinum-loopful on the surface of agar. After a night's incubation, a film of growth studded with bare plaques will have appeared. Now if one of these is touched with the tip of a platinum needle which is then dipped into a suspension of sensitive bacteria, the phage will be regenerated; again very often with a restricted range of potency. This procedure is similar to the common method of isolating pure cultures of bacteria, and d'Herelle believes the plaque to be a colony of the ultramicrobe Bacteriophagum intestinale. The rival school, on the contrary, pictures it as a focus of autolysis starting from a cell or group of cells having a specially strong affinity for the bacteriophage.

The potency of a lytic filtrate for a given bacterial culture is estimated by counting the plaques produced by a unit volume of a known dilution. The appropriate dilution has to be found in a preliminary experiment, since too big a dose may completely prevent growth by producing an infinite number of confluent plaques.

When a *highly active* bacteriophage is tested in serial dilutions on a single sensitive race of bacteria, the number of plaques varies with the concentration of the phage, and is independent, within a wide range, of the number of the bacteria on which it is acting. This most important fact is well established and constitutes one of the foundation-stones of the virus-theory; for the simplest explanation is that the bacteriophage is corpuscular, and that each corpuscle produces a plaque. Nevertheless, the following observation of Bordet throws doubt on this interpretation. A bacteriophage active for both *Bact. coli* and *Bact. dysenteriae* (Shiga) was added in equal concentration to similar suspensions of the two microbes separately. A sample of each mixture was spread on agar and incubated; with the result that growth of *Bact. coli* was entirely lysed, whereas the Shiga culture showed merely a limited number of plaques in a good film of growth. If, then, there were only enough bacteriophage corpuscles to cause a limited number of plaques in the one culture, how could they give rise to innumerable confluent areas of lysis in the other? It is much more likely that the different behaviour of the two species depended on their different susceptibilities. In the one practically all the cells, in the other only a few were sensitive.

In experiments with 'weak' phages the number and size of plaques vary quite obviously with the number of bacteria as well as with the concentration of the lytic principle. They are also greatly affected by the composition of the agar-medium, being larger and more numerous on soft than on harder (more concentrated) agar. With such phages the estimates of potency made by the two methods, dilution and plaque-counting, do not necessarily tally; so that the number of hypothetical bacteriophage corpuscles cannot be arrived at with any certainty.

Regarding the relation between ' strong ' and ' weak ' phages, Bordet made the following significant observation. If a small amount of a phage of high potency is added to a bacterial suspension of moderate density and a drop removed in the early stages of lysis, the resulting phage may show a much-lowered potency, and this lowering persists unaltered in subsequent passages. Now this cannot be explained at all by the virus-theory, but is intelligible if the phage is a cell-product ; since the first cells to be lysed are the least resistant ones, and cells of low resistance can only produce a ' weak ' lysin, i.e. one whose affinities are restricted to ' weak ' cells.

The Significance of the Size of Plaques. The bare areas of lysis on solid media are circular and generally of a fairly constant size. They do not usually spread when once the culture is fully grown. Sometimes, however, they are surrounded by a semi-transparent ring of incomplete lysis ; and when the attempt is made to propagate the phage from this area, either no lysin is obtained or a weak one which fails to regenerate. This phenomenon has been taken to prove the diffusion from the plaque of a non-transmissible soluble lysin secreted by the bacteriophage, but since weak phages are often difficult to propagate, no such explanation is necessary.

The plaques formed by some phages in certain cultures vary considerably in size, and careful analysis has shown that the variation is not haphazard, but regular, and that there are two main classes, large and small.

On recovery of the phage from a small plaque, it is found to breed true, that is, to produce, in the same species of bacteria, only small plaques. But the large-plaque phage, after producing at first only large plaques, splits up, just like the parent phage, into ' large ' and ' small '. The species of microbes in which this phe-

nomenon has been observed are the paratyphoid and dysentery bacteria and *Proteus*.

When cultures showing the two plaque-types are incubated further, secondary colonies appear on some of the plaques; and, strange to say, those growing on the large ones are Rough, and those on the small Smooth (see p. 30).

The explanation is clear: The culture is producing Rough and Smooth variants and the phage is a mixture of two lytic principles, to one of which the Smooth elements are susceptible, and the Rough resistant; while the other lyses the Rough phase, and does not touch the Smooth. The scanty residual living cells in a plaque are those with little or no affinity for the type of phage that is being regenerated in that plaque. This is readily corroborated by isolating each of the phages in a pure state from one of their respective plaques and testing them on pure Smooth and pure Rough bacterial variants.

This phenomenon of restricted affinity undoubtedly accounts for the partial action of many of those phages that are called 'weak' or of 'low virulence' by d'Herelle and his followers.

We have seen that the large-plaque phage (lysing Smooth) under appropriate conditions generates both 'large' and 'small', whereas 'small' can only reproduce itself. For an ultramicrobe, this would be very mysterious behaviour; but for a cell-constituent, closely connected with the variant antigens, it is natural enough. For we have learned (p. 32) that the Smooth bacterium is relatively impermanent; tending to split into Smooth and Rough; whereas the latter, once formed, is commonly more stable.

The explanation of the greater size of plaques caused by the 'Smooth' phage is that Smooth bacteria react with greater velocity to their own special lysin than the

Rough do to theirs. In a mixed culture, therefore, the 'Smooth' plaques form earlier and spread more in the time at their disposal. Both cease to enlarge as soon as the culture has reached the end of the logarithmic phase of growth.

We can summarize this section by saying that '*Potency*' *expresses the range of affinity of a bacteriophage for the variable units in a bacterial population, and is determined by the constitution of the cells in which the phage has been regenerated.*

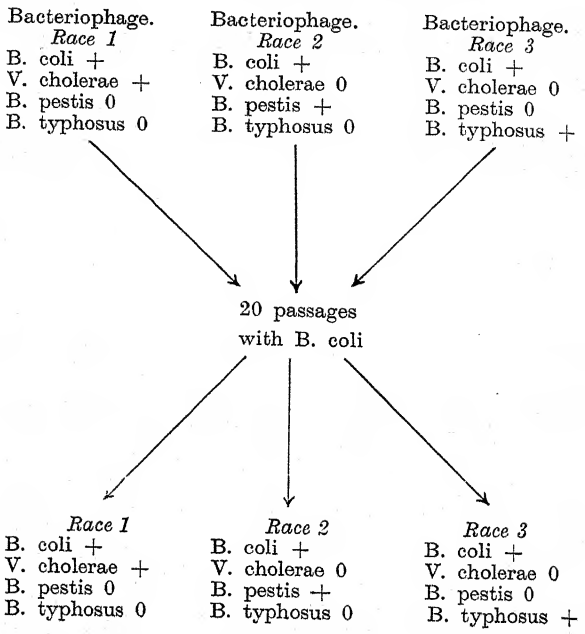
§ 7. THE INDIVIDUALITY OF BACTERIOPHAGES

It has already been mentioned that every strain of bacteriophage has a characteristic range of actions on diverse, but generally closely connected races of bacteria. This individuality is maintained with a certain tenacity, as is shown in the diagram on page 98, which illustrates the maintenance of specific ranges of action on various cultures by three distinct bacteriophages after twenty passages (cultivation-lysis-filtration) through cultures of the same race of *B. coli* (d'Herelle, 1930).

To quote from Burnet (1930):

'Different phages developed at the expense of a single strain can be characterized in several ways; by their activity against other bacteria, their power to provoke qualitatively specific resistant strains, their resistance to heat, their power to induce specific antilysins on injection into animals and by the size and appearance of their plaques. Such specific qualities have regularly been found to persist through successive passage on one strain and in most cases even when the phage is grown at the expense of some different bacterial species. . . . It is undoubted that changes in phage character may appear after passage under certain conditions, but these occasional modifications are much less impressive than the constancy of character which is the general rule. There is only one simple explanation of this constancy—that the successive crops of phage-particles are genetically related.'

This author is attracted by the simplicity of the virus



theory and doubts the ability of any other theory to explain the facts without formidable complication. We feel, however, that the simplicity is spurious and the complexity overrated. We know, in fact, that the antigenic structure of bacteria and the relationships of the antigens of different species are complex, and therefore that any series of cell-constituents derived from a range of cultures is equally bound to be heterogeneous, and to have complicated affinities, whether the constituent is of the nature of an enzyme or a genetic unit. There is, indeed, no agreement as to the direction in which the individuality of bacteriophages really points; and we are free to pursue our line of reasoning by the assumption

that *it is the structure of the antigenic complex, with which regeneration is involved, that determines and perpetuates the individuality of the lytic principle.*

§ 8. THE CHARACTERS OF SECONDARY CULTURES

The degree of resistance of secondary cultures to the particular phage employed depends on the dose with which the primary culture was treated. A dose large enough to cause almost complete lysis gives rise to a highly resistant secondary growth. If a small dose is used, some of the cells, when spread on agar, will grow into normal, sensitive colonies. According to the virus-theory, these cells have escaped infection; but in our view they owe their survival to a temporary modification in the direction of resistance, their subsequent progeny returning, according to the well-known biological law, back to the mean.

Other cells of this secondary culture are completely resistant to the phage employed, though of course not necessarily to other phages. Moreover, filtrates of cultures from such cells have no lytic action on sensitive sister-cultures. Finally, some colonies are resistant but lysinogenetic. That is to say, they propagate the phage without harm to themselves. The presence of the lytic principle is demonstrated by its action on a sensitive culture of the same or other strains. The virus-theory interprets this as symbiosis; the opposite school as a proof that liberation of the active material does not depend on destruction of the cell, and that resistance is not inconsistent with the possession of some slight affinity for the phage.

The secondary growth occurring either in a liquid or on a solid medium after the destruction of all the susceptible cells by bacteriophage is often delayed for

several days or even weeks. This suggests that the surviving cells have been damaged to a certain extent, and need time to recover. Further, the cultures often show morphological abnormality, the cells being short and spheroidal. There is a strong tendency for resistance to dwindle away, in which case the shape of the cells also returns to normal. The significance of the change is not known; but it is in any case not peculiar to the bacteriophage-phenomenon, since similar variations are encountered under other circumstances.

In *Bact. coli* a capsulated variant appears not uncommonly as the resistant secondary growth. In such cases it is not clear whether the capsule acts mechanically, in which case we have here a special form of resistance, not due to lack of affinity; or whether it is a mere alternative to the Rough-resistant form, and has the same antigenic construction. According to d'Herelle, lysinogenetic secondary colonies which contain resistant cells and active bacteriophage are small, viscous and of slow growth.

Some species of bacteria do not seem to throw off completely resistant variants. In the Flexner group of dysentery bacilli, for instance, the strongest secondary growths usually show numerous moth-eaten colonies. It is perhaps significant that the normal Smooth phase is on the whole more stable in the dysentery bacteria than in most of the species of the great typhoid-coli group, and that the Rough (resistant) phase is often very hard to procure.

Other variations that have been attributed to the action of bacteriophage concern pigmentation, mobility (growth of flagella) and various biochemical functions, including virulence. Most of these are merely concomitants of the Smooth to Rough transformation, and none of them differ essentially from the spontaneous variations

we have described in our first section. If then, the bacteriophage is an important cause of the variation of cultures, we may conclude that it acts by the eliminative selection of preformed variants.

The Alleged Ultramicrobic Phase of Microbes. A number of workers have reported a slow growth of bacteria in filtrates of bacteriophaged cultures, and have deduced from this that the bacilli give rise under the action of the phage to invisible forms of the size-order of viruses, which pass through the filter and reproduce the original culture. We have already mentioned the matter in Part I (p. 14), and have seen that the same phenomena have been described as occurring without bacteriophage action (see Hauduroy, 1929). There is nothing to add here to the warning already expressed against the deduction of revolutionary principles from complicated data obtained by methods which are by no means proof against gross error.

§ 9. ANTIBACTERIOPHAGE SERUM

Filtrates of lysed cultures when injected into animals produce an anti-serum that inhibits the action of the phage. There is good evidence that an antibody is produced to the lytic principle itself, in addition to the inevitable one for the bacterial matter in the filtrate ; but the conditions are complex and the experiments difficult to interpret. For further information the reader is referred to Burnet's summary in the System of Bacteriology (1930).

§ 10. THE RÔLE OF THE BACTERIOPHAGE IN INFECTIOUS DISEASES

D'Herelle's observations on the transmissible lysin, and more especially his belief that it is a devastating disease of bacteria, led him to put it to the proof as a remedy for bacterial infections of man and animals. At

the same time he made a large number of observations which convinced him that spontaneous recovery from infection is due not to immunity involving tissue changes and the production of antibodies, but to the adaptation of an already present bacteriophage to the infecting

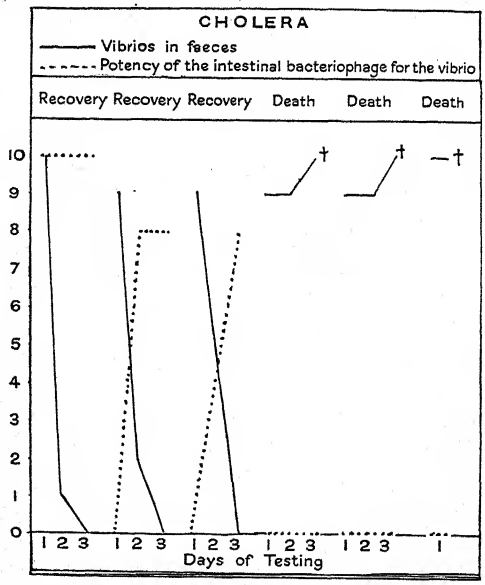
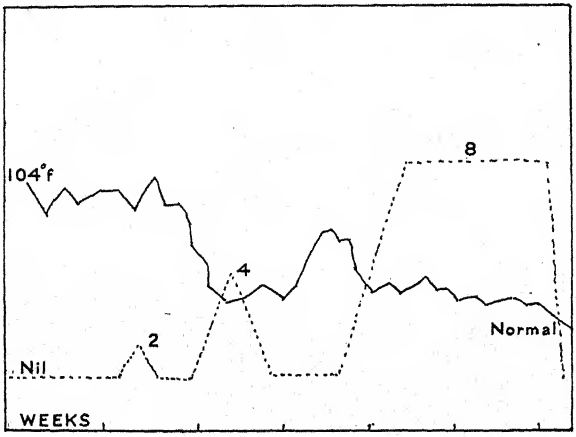


FIG. 20.—After d'Herelle, 1930

bacillus and to the consequent elimination of the latter. The course of an epidemic thus reflects a struggle not between the human or animal host and the microbe, but between the latter and its ultramicrobic parasite. In cholera, typhoid fever and plague spontaneous recovery was shown to be accompanied by (1) Disappearance of the infecting bacteria and (2) Increased activity of the bacteriophage for those bacteria (Figs. 20 and 21).

Details may be found in d'Herelle's book (1930). If further experience bears out his main contentions there is no doubt that our views of infective processes will have to be radically changed. At present, however, the position is obscure, owing to the failure of many experienced workers to confirm d'Herelle's main contentions.



——Temperature of Patient. .....Potency of Bacteriophage.

FIG. 21.—Graphs of Temperature and potency of Bacteriophage in a case of Typhoid Fever. (After d'Herelle)

A complete account of this aspect of our subject would be out of place here, but we will illustrate its main points as shortly as possible, beginning with a quotation from d'Herelle (1926) concerning some investigations on rats as carriers of the plague-microbe in India.

'In Bombay, where plague is endemic, between 30 and 99 per cent of the rats are refractory (to artificial infection), the percentage varying with the season. Avari tried to find a bacteriophage for *B. pestis* in the rats of Bombay, but did not find it. These rats have thus a true immunity.'

Note the argument: true immunity means a native

insusceptibility apart from bacteriophage action. This could only be acquired by exposure of the race to constant infection. Since these rats had no bacteriophage, their resistance could not be due to it. But now :

' At Madras, a district free of plague, 100 per cent of the rats are susceptible.' . . . ' All of the Madras rats inoculated with plague bacilli contracted the disease, and occasionally a rat, one out of each 200 or 300 inoculated, after having been manifestly sick, recovered. Avari sacrificed these recovered rats, and constantly found in the intestine and spleen a bacteriophage possessing a high virulence for *B. pestis*. This he never did in those which died.'

Here, then, we have a breed of rats that has not been exposed to plague infection, and therefore remains susceptible. Nearly all die of infection without developing any phage ; a few survive and contain phage. ' These experiments show very clearly that the process of recovery in plague is the same as that in the intestinal diseases ' (i.e. by bacteriophagy). Recovery, in d'Herelle's view, is a phenomenon entirely distinct from immunity, which develops as the result of recovery, but does not participate in it. Recovery is caused by phage, immunity results from recovery.

In an epidemic, an individual in the process of recovery acts as a centre of spread of the bacteriophage, which has worked itself up to a pitch of great ' virulence ' for the microbe. We thus arrive at the novel and attractive notion that just as we catch infection, so we catch disinfection.

In the application of these ideas to the treatment of the serious intestinal infections, cholera, dysentery and typhoid fever, d'Herelle reports dramatic and unbroken success. All that is necessary to bring an individual case or an epidemic to a happy conclusion is to apply the right bacteriophage in the right way. Failure comes from the use of an inappropriate weak phage, to which

the bacteria rapidly become resistant; in which way more harm than good is done. When cholera was ravaging the villages of the Punjab, d'Herelle, at the outbreak of an epidemic in a village, poured some potent bacteriophage into the wells. In every case but one the epidemic ceased as by a miracle, and when the one failure was more carefully looked into, the discovery of a secret well soon put things right.

So great, again, has been the success of the treatment in bacillary dysentery in Brazil that it is said to be the only therapeutic method for that disease now practised there.

A great deal of work on these lines has been, and is being done in all parts of the world, and it must be confessed that the results are puzzling. Some workers are enthusiastic, others cold. Many of the latter have clearly not followed d'Herelle's directions to the full, but on the other hand careful work, especially by English-speaking bacteriologists, has often given most disappointing results. Further, when the method has been tested on artificially infected animals under experimental conditions, as a rule no favourable effect has been established. Only when the bacteriophage and the bacteria are administered at the same time is the effect of the latter apparent. It is important to realize that the phage cannot be administered in a pure state, for the fluid is invariably rich in the products of bacterial disintegration, and since these 'antigens' have a strong immunizing power—since in fact they are a form of 'vaccine'—the state of affairs is far from simple. Dissolved vaccines without any connexion with bacteriophage, have been widely and successfully used in the same range of infectious conditions. Moreover, anybody acquainted with disease knows how extremely difficult it is to assess the effect of a remedy. As Martin Arrowsmith sadly discovered, it is next to impossible to handle

the matter scientifically where human beings or valuable animals are the subject. One may plan to administer a promising remedy in 500 cases, and withhold it, for comparison, in 500 others; but so soon as it appears to be at all successful, all must have it. Moreover the recovery-rate from all infections is high—usually very high indeed—and varies from moment to moment. Epidemics must always regress sooner or later, or the affected species would rapidly die out, and how are we to know that our treatment has not been started on a falling curve? Such considerations oblige us to scepticism, just as the enthusiasm of d'Herelle and his supporters, and the profound importance of their work, demand our sympathy and support. It may be that the bacteriophage, whether it is or is not an ultramicrobic parasite of bacteria, will prove a powerful weapon in our unceasing war against the microbes.

Apart from its lytic action, the phage exercises on bacteria two other effects that might be calculated to assist the infected organism. First, bacteria treated with the phage are ingested by phagocytes more readily than normal bacteria. In other words, the phage has an opsonic action. Second, it has been emphasized by Hadley that what he calls the 'dissociative' action of bacteriophage, by which he means its power of turning virulent smooth cultures into the benign Rough phase, may be as important as its lytic effect. There is, however, practically no evidence that this phase-change commonly occurs in the stage of recovery from infection, or that, in the few cases when it has been demonstrated (e.g. diphtheria) it has been caused by the bacteriophage.

§ 11. ANIMATE OR INANIMATE?

All known living things have a recognizable form or structure; they assimilate matter, grow, multiply and

maintain their identity by adaptation through a certain range of alterations of their environment. It is more than doubtful, however, whether this description constitutes a definition of life. Indeed we may well be wrong in searching for a sharp line of division between the living and the lifeless. Biology is rich in instances of semi-living things such as skeletal structures, erythrocytes and chloroplasts. Moreover, we have lives within lives; the life of the organism, and that of the cell. Carrel's chicken died twenty-five years ago, but a fragment of its tissues is alive to this day. Within the cell are the inexplicable mitochondria, and the chromosomes, which have form, grow and multiply. Within the chromosomes the invisible gene can be inferred, named and even assigned to a position. It, again, seems to maintain an individuality—a sort of limited independence. If the bacteriophage could be proved to be an enzyme, would that remove it from the category of the living? Enzymes or catalysts must certainly be an essential part of the vital mechanism of the cell, just as cells are the building-material of higher organisms. We doubt, therefore, whether any real light can be thrown on the nature of bacteriophage, or even of viruses, by the attempt to place them on one side or other of a probably non-existent boundary-line.

Nevertheless, since the virus-theory emphasizes strongly the living nature of the 'Bacteriophagum intestinale', classing it as a species of Protobe, consisting of individual Protules, it is desirable to examine the reasons for this view, in so far as they have not already been given elsewhere.

The main arguments are three: (1) The bacteriophage is particulate. (2) It assimilates. (3) It is capable of adaptation.

We have already dealt with the first argument, and

have concluded that it remains unproved. For even though the active principle behaves in some respects as a particle, this may well be due to its absorption on colloidal aggregates such as are always present in culture-fluids.

Assimilation can only be proved by demonstrating that within a system composed of the organism and assimilable substances the mass of the former increases at the expense of the latter. But in the case of the bacteriophage we cannot arrange a simple nutritive system, owing to the inability of the phage to multiply in the absence of living bacteria. It is true that certain workers have claimed to demonstrate some multiplication in a sterile solution containing the soluble products of bacterial metabolism, but this cannot at present be accepted. So long, in fact, as living cells are present, one cannot exclude the possibility that the phage is made by the cells, and even less can a metabolism of the phage 'corpuscles' be deduced, as d'Herelle deduces it, from the bare fact that a progressive increase of phage activity occurs.

The question whether the bacteriophage shows any biochemical activity has been approached from another angle. Respiration, for instance, has been investigated by seeking for an output of CO_2 in lytic filtrates free from bacteria. None, however, has been observed. A fluid containing 10^{12} units of active bacteriophage was tested in a micro respirometer for ten days, with an entirely negative result. Now the spores of bacteria definitely respire under similar conditions, and since the phage unit is far smaller than a spore, its respiration, if it had any, should be relatively much quicker. But it can be calculated that the respiration of 10^{12} spores would have to be retarded to a ten-thousandth part of its normal rate for CO_2 not to be detected in ninety-six

hours; and it may therefore be deduced with considerable probability that the bacteriophage does not respire at all. Again, the rate of oxygen consumption in bacterial cultures has been repeatedly measured during the process of bacteriophagy, and it has been established that it depends only on the number of living bacteria, and in no way upon the concentration of the bacteriophage (see Bronfenbrenner, 1928).

Thus d'Herelle's opinion that assimilation is a proved property of the bacteriophage from which one can deduce its living character cannot claim any direct experimental support.

Adaptation. We have already mentioned that lytic activity of a bacteriophage can often be widened in range by offering to it bacteria on which it has at the outset no evident action. It is probable, though not proved, that the races of bacteria for which it can thus become active are restricted to those possessing an antigen in common with those on which the phage is normally propagated. The biological explanation of these phenomena as 'adaptation' is certainly attractive, but is less definite than it sounds, since it makes no attempt to describe the mechanism of the change. Moreover, the living bacteria are always present, and it may be that they are responsible.

Clearer evidence has been sought along different lines by Prausnitz and others, who added chemical substances such as phenol, mercuric perchloride and chloramine to mixtures of bacteria with an active phage, in concentrations too low to inhibit seriously the bacterial growth (see Table II).

After a series of passages through such cultures, the phage was finally exposed to the same concentration of the antiseptic for twenty-four to forty-eight hours in the absence of bacteria. A sample of the same phage

propagated an equal number of times without antiseptic was similarly exposed, and the potency of the two was compared at intervals by the method of plaque-counting. The results of two such experiments may be quoted :

EFFECT OF EXPOSURE OF BACTERIOPHAGE 9 TIMES TO 1 IN 10,000 SOLUTION OF PERCHLORIDE OF MERCURY

		Number of Plaques	
		After 1 day in $HgCl_2$	After 2 days in $HgCl_2$
Bacteriophage exposed 9 times to $HgCl_2$	$HgCl_2$ 1/10000	c. 2,000	370
	Physol. Saline	c. 3,000	410
Same bacteriophage not exposed to $HgCl_2$	$HgCl_2$ 1/10000	0	0
	Physiol. Saline	454	175

EFFECT OF EXPOSURE OF BACTERIOPHAGE 22 TIMES TO 0·75 PER CENT PHENOL (VARIATION OF PLAQUES)

	No. of Plaques after 48 hours in	
	0·75 per cent Phenol.	Water
Exposed Bacteriophage Plaque I . .	632	768
Plaque II . .	82	554
Untreated Bacteriophage	0	578

There can be no doubt that the treated phage resists the action of the antiseptic far better than the untreated, and that if the lytic principle is an ultramicrobe, the explanation of this phenomenon as an adaptation is perfectly reasonable. Nevertheless it is by no means impossible that the change in the phage is due to a selective action of the antiseptic on the bacteria in each separate passage. The cells most sensitive to the antiseptic would be inhibited, and the full-grown culture would therefore be altered by selection in the direction

of greater resistance. If the phage originates as part of the living substance of the cells, might it not be expected to share in their special properties ?

So long as the bacteriophage cannot be propagated in a lifeless medium, no real proof of its living nature seems possible. If ever this propagation is achieved, no further proof will be required.

The ' Homogamic ' Theory of Hadley. This may be classed as a third variety of the theory of cell-origin. Convinced that the dual action of the phage on the Smooth and Rough phases of a culture is inconsistent with the nature of an independent ultramicrobe, and dissatisfied with all other explanations, Hadley turns to the mysterious realm of ' bacterial cyclogeny '—a term which implies that there is more in the life-history and reproductive mechanism of bacteria than conservative bacteriologists are prepared to admit.

He postulates the existence of an ultramicroscopic phase of bacterial growth with the following remarkable properties : (1) It incites the cell not only to proliferation, but also to disintegration. (2) It increases ' at the expense of the process it instigates '. (3) It possesses ' some sort of fecundating significance ', i.e. it must be able to effect a conjugation with, or perhaps a fertilization of, the young susceptible cells. Hadley disarms criticism of this rather bewildering repertory by admitting that he is indulging in speculation. But does it really help us ?

We have seen in our first section that bacteriologists as a whole, including most of those who have made a special study of the matter, do not believe either in sexual processes or in the ' cyclogeny ' which entails an ultramicroscopic phase. Clearly, then, they cannot accept an explanation of the bacteriophage based on the reality of such phenomena.

§ 12. VIRUS AND BACTERIOPHAGE

The bacteriophage phenomenon, taken by itself, is explicable as a metabolic disorder transmissible by means of a free catalyst, the variable nature of which is determined by the cells in which it originates. It is therefore neither necessary nor desirable to endow the active agent with independent life. Yet obvious resemblance of the bacteriophage to the viruses, which are generally accepted as living ultra-microbes, has biased many minds towards the ultramicrobic nature of the bacteriophage.

We are, however, under no obligation to believe that the viruses form a homogeneous group of phenomena. It is admittedly very probable that the majority are living organisms of extreme minuteness, for their behaviour resembles closely that of bacteria, even to the type of reaction (inflammation) to which they give rise in animal tissues. Moreover, there is no dividing line of magnitude between bacterium and virus, for the gap is bridged by what we have called the border-line organisms.

Certain viruses, however, may well be of a different order of being. The agents of *Molluscum contagiosum* and of the various fowl-tumours cause a distinctive type of tissue-reaction. Instead of inflammation and cell-degeneration, we have the formation of new-growths of a constant histological structure, which lead us directly into the obscure though fascinating realm of cancer and tumour-formation in general. Since the accepted principles of Pathology and Genetics fail to give any explanation of the transmission of an abnormal cell-structure by means of an invisible agent, the hypothesis of externally transmissible type-determining factors (? genes) must be given serious consideration. Now Bordet, in his theory of bacteriophagy, suggested that the autolysis

might be an indirect action; the transmissible agent being rather an inciter of catalyst-production than an actual catalyst. Wollman, Bail and others are more definite in regarding it as an ultramicroscopic structural unit of the disintegrated cell capable of entering other cells and endowing them with the abnormal quality that it carries.

The phenomena viewed in this way have a striking similarity with the behaviour of the infective fowl-tumours, and together they constitute a strong argument for the theory of externally transmissible genetic factors, the analogy with which will be further considered from a more general point of view by Mr. de Beer in an appendix.

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APPENDIX

By G. R. de Beer

THE ANALOGY BETWEEN THE BACTERIOPHAGE AND THE MENDELIAN FACTOR, OR GENE

IN 1922 attention was drawn by Muller to the analogy which exists between the properties of the Mendelian factor or gene, and the Twort-d'Herelle phenomenon or bacteriophage. To begin with the gene, a body of evidence which is now irrefutable, demands that the phenomena of Mendelian inheritance in animals and plants be regarded as controlled by genes, conceived as particulate structures associated with the chromosomes of the cells. The genes are capable of self-propagation, and since it is the rule that daughter cells are found to possess genetic qualities identical with those of their parent cell, there must be a synthesis of the gene-substance prior to every cell-division. The genes are also capable of change or mutation, and they are then capable of self-propagation in their changed condition. The genes by their interaction with one another and with the environment control the physical characters of the organism, and a changed or mutated gene is the basis of inherited variation. Of their dimensions it is difficult to form an accurate opinion, but it is significant that three estimates, by independent lines of approach, approximate to the same order of size, viz. 20, 60 and 77 milli-micra. The gene is, of course, an intra-cellular structure, but it has recently been shown by Sturtevant that the effect of a gene can be manifested in adjacent cells which themselves do not possess that gene. This can only be inter-

preted to mean that the gene is capable of acting intercellularly.

Now, every one of the properties described above with which the gene is endowed, is paralleled by those of the bacteriophage, except for the association with chromosomes. The bacteriophage is capable of self-propagation, and, most significantly, this bacteriophage-synthesis only takes place when the bacterial cells divide. Further, the bacteriophage is capable of change and is then self-propagated in its changed condition. That the bacteriophage is related to the phenomena of variation emerges from Bordet's demonstration that 'weak' bacteriophage working on a culture of the 'Smooth' type of *Bact. coli* results in abundant production of the 'Rough' type, which is of course immune to the weak principle. The 'weak' principle therefore represents only a more active form of a substance which the 'Smooth' type produces normally, and which has the power of making the other 'Rough' type appear in its stead. The order of size of the bacteriophage is given as 8 to 30 milli-micra, which agrees remarkably well with the dimensions advanced for the gene. As for the intercellular nature of its action, the bacteriophage has been regarded as 'an ultramicroscopical structural unit of the disintegrated cell that can enter other cells and endow them with the abnormal quality that it carries', to quote Dr. Gardner's penultimate paragraph. The bacteriophage may then be held to be analogous with an abnormally abundantly developed gene with lethal effects which it exerts intercellularly.

Admittedly, of course, the analogy between the gene and the bacteriophage is nothing more than an analogy, and has no evidential value. But the terms with which it deals are of such interest and importance, that it seems well worth while drawing attention to its existence and attempting to probe more deeply into it. If it should turn out to be a good analogy, the geneticist will then be able to study these self-propagating substances in a manner in which he is at present unable to do within the cell. As Muller has picturesquely put it, it may after all not be

impossible to grind up genes or their equivalents in a mortar, or boil them in a test-tube.

On the theoretical side, much interest attaches to the manner in which self-propagation of the bacteriophage is envisaged by Bordet, whose theory seems to be much the most suitable. The bacteriophage would 'grow', not by assimilation of foreign matter and building it up into matter like itself, but by stimulating something else—the cell—to synthesize more matter like itself. Applying this view to the gene, there is no necessity to grapple with the difficulty of understanding how the gene, a complex molecule or even group of molecules, can be divided so accurately that the genetic properties of sister-cells are identical. Instead, the gene would be regarded as endowed with the property of stimulating the protoplasm of the cell in which it is situated to synthesize more genes identical with itself, and these would then be equally distributed and not divided at cell-division. The possibilities of variation in the processes of synthesis under changed conditions could be invoked to account for the production of mutations. But it is idle to speculate further, and the sole purpose of this appendix is to point out that the science of genetics, which has derived such great advantage from its alliance with cytology, may not be ill-advised to court the help of bacteriology in the solution of one of its major problems.

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GLOSSARY OF TECHNICAL TERMS NOT EXPLAINED IN THE TEXT

Absorption (of antibodies). An antiserum is mixed with a dense suspension of bacteria, incubated for some hours and centrifugalized till clear. The specific antibodies combine with the bacteria and are thus removed from the fluid.

Acid-fast. Retaining the dye after staining with carbol-fuchsin and treatment with 25 per cent H_2SO_4.

Agar (or agar-agar). A gelatinous material extracted from a seaweed and used as a basis for solid culture-media. Not nutritive in itself.

Agglutinin. An antibody which will react with a suspension of bacteria containing the specific antigen and cause them to aggregate into clumps.

Antibody. A substance in, or a property of, blood or other body-fluids of an animal that has been injected with an antigen or infected by a microbe containing the antigen. By virtue of this substance the fluid reacts with the antigen, neutralizing, precipitating or agglutinating it, or fixing complement with it. See complement-fixation.

Antigen. A substance which, when injected into an animal, causes the production of a specific antibody.

Bacteria. Microbes. Schizomycetes.

Bacterium. A genus of Schizomycetes, often, loosely, a microbe.

Broth. An infusion of meat with added peptone.

Cancer. A malignant, penetrating and disseminating new-growth of atypical cells.

Cataphoresis. An electrical method of separating out particles in a fluid, taking advantage of their electro-positive or negative properties.

Complement-fixation. The power of combined antigen and antibody to prevent the haemolytic or bactericidal action of 'Complement' (a hypothetical substance in fresh blood-serum).

Culture. A population of bacteria growing in a single receptacle (but often used in sense of 'race').

Dysentery. A severe bloody diarrhoea caused by (1) bacteria, (2) amoebae.

Epidemic. A disease infecting a population of man or animals with a tendency to periodic recurrence.

Endemic. A disease established in a population, manifesting itself sporadically in individuals, without periodicity.

Generation. In bacteria: a single fission, producing two cells from one.

Inoculate. To inject bacteria or their products into any part of an animal, or even into a tube of culture-medium. An absurd term derived from an early practice of injecting into the eye.

Lysinogenetic. Giving rise to a substance with dissolving power. Usually refers to bacteriophage.

Microaerophilic. Growing best in low oxygen-pressures.

Microbes. Microscopically visible bacteria.

Modification. An evanescent divergence from the mean.

Mutation. A sudden variation proving fixed or hereditary.

Opsonic. A property of serum or other body-fluids which renders bacteria more susceptible to ingestion by wandering white blood-corpuscles (phagocytes).

Plating. Spreading a small quantity of a suspension of bacteria on the surface of a gellified culture-medium, to obtain separate colonies.

Precipitation. The clouding or flocculation of an antibody-containing serum when mixed with a colloidal solution of an antigen.

Psychrophilic. Liking an unusually low temperature.

Race. Any series of sub-cultures from a single source.

Sarcoma. A malignant new-growth of cells of the connective-tissue series. A form of cancer.

Saprophytic. Thriving in a lifeless environment (opp. of parasitic).

Serological. Appertaining to the study of antiserums (antibodies).

Subculture. Any new culture made from an existing one.

Thermophilic. Liking an unusually high temperature.

Toxicity. Poisonousness of living or dead bacteria.

Ultramicrobes. See Introduction.

Vaccine. A suspension of microbes or ultramicrobes used for injection into man or animals.

Virulence. Power of causing disease by invasion, multiplication and toxicity.

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